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447. MILK YIELD IN RELATION TO INFECTION WITH *STREPTOCOCCUS AGALACTIAE*

BY D. H. McLEOD AND S. M. WILSON
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Two incompletely solved problems concerning infection in the bovine udder by *Streptococcus agalactiae* are: (i) whether susceptibility to infection is, or is not, associated with yielding capacity; and (ii) the effect of infection on milk yield. On both questions varying opinions are often expressed, and there is a need for definite evidence.

Four tuberculin-tested herds, which had been examined for udder infections at approximately 3-monthly intervals for periods of 9–13 years between 1935 and 1948, appeared to provide data suitable for an investigation of both problems. Records of the milk yield and of the occurrence of infections by *Strep. agalactiae* have now been analysed, and the results are described in this paper.

METHODS

Infected cows. The methods of sampling and bacteriological diagnosis have been described previously (1). The criterion of infection adopted was the isolation of *Strep. agalactiae* from quarter samples on at least two successive occasions. Since for problem (ii) it was desired to find the overall effect of infection, irrespective of the severity of attack, no attempt was made in this work to classify infections according to the number of quarters involved or to the incidence of clinical mastitis. The milk yields of the cows after they acquired infection were obtained for only the lactation in which infection was detected, second lactations of infection being usually unobtainable since an eradication scheme was in operation in all four herds.

Methods of control. Any comparison of yields is difficult because of the large number of factors which can affect milk production. In this study the method of control employed was to use as controls for each infected animal only those uninfected animals in the same herd whose lactations commenced within 4 months of the start of each of the corresponding lactations under examination in the infected animal. The average yield of each group of controls so selected was used as the control yield, to be compared with the yield of each corresponding individual infected animal. In this way for each infected animal there was eliminated variation due to age, year, season and herd management.

Milk records excluded. Records which were excluded from this analysis were those for animals recorded for one lactation only, those for animals which became infected in their first lactation, or which were found to be infected when first tested, those of lactations with blind quarters, those of animals suffering from Johne's disease, those of animals treated with sulphonamide or penicillin, after treatment had been given, and those of farrow lactations. Other records had naturally to be excluded because of incompleteness or because of the lack of suitable controls.

Subsidiary factors examined. A preliminary examination was made of other factors which might have had an effect on yields. The animals were divided into two groups, those which became infected with *Strep. agalactiae*, and those which did not. The two groups

were then examined for the incidence of infection with *Strep. dysgalactiae*, *Strep. uberis*, group G streptococci, haemolytic staphylococci, *Corynebacterium pyogenes*, *Brucella abortus* and clinical symptoms of unknown aetiology. These factors were considered separately and then together, but no significant difference was found between the two groups. A similar examination was made for breed distribution, length of service and dry periods, and again no definite difference was detected between the two groups.

MILK YIELD AND SUSCEPTIBILITY TO INFECTION

Although 268 animals were found to be infected with *Strep. agalactiae* in the four herds over the period of examination, the records of only fifty-seven animals were suitable for this analysis because of the selection entailed by the methods of control. The average lactation yield of each infected animal, over all the lactations preceding the one in which infection was first detected, was compared with the mean of the average lactation yields of its controls in their corresponding lactations. The difference between the two figures indicates how much more or less the infected animal had yielded prior to infection than did its controls in the corresponding period. (In the following a positive figure denotes that the infected animals yielded more, a negative figure less, than the controls.) The variability was very large, from +412 gal. to −371 gal. per lactation, thirty-five of the infected

Table 1

Herd	No. of infected animals	Average difference (gal./lactation)
A	4	+69·8±78·1
B	9	+102·7±52·1
C	15	+30·2±40·3
D	29	+53·9±29·0

Table 2

Lactation in which infection occurred	No. of infected animals	Average difference (gal./lactation)
2nd	21	+81·9±32·2
3rd	17	+31·6±35·8
4th	8	+150·0±51·2
5th	6	+25·5±60·3
6th	5	−78·4±65·0

animals yielding more, and twenty-two less, than their controls. Before an average figure for all the animals could be considered, it was necessary to determine whether there were any differences between herds or between cows that became infected at different ages (in lactations). The herd averages were as shown in Table 1. There were no significant differences between the different herd averages. The lactation averages were as shown in Table 2. Since the variability among the lactation averages was high, and since there was no detectable trend with advancing age, it is probable that the large differences between some of the averages were chance effects.

The average difference for all the fifty-seven animals was +56·5 gal./lactation, with a standard error of 20·37, which gives a figure of 2·78 for t and about 0·01 for P. The mean of the average lactation yields prior to infection was 725·8 gal. for the infected animals, and 669·4 gal. for the controls in the corresponding periods, the infected animals having thus yielded 8·4% more than the controls.

As it was possible that this difference between the infected animals and the controls might have existed only in the period immediately preceding infection, a further comparison was made, using in this case the yields given in the lactation immediately before that in which infection occurred. The average difference between the infected and control yields was then $+49{\cdot}0 \pm 23{\cdot}2$ gal./lactation ($P = 0{\cdot}035$). There is obviously no significant difference between this and the previous figure, suggesting that the difference noted was spread over the whole milking life of the animals. On the other hand, it should be noted that in a large proportion of the cases (the animals infected in the second lactation) the same figures had to be used for both comparisons.

THE EFFECT OF INFECTION ON MILK YIELD

In this part of the investigation a further selection of the infected animals was made, since only in those animals infected relatively early in the lactation period could infection be expected to have had an appreciable effect on the total lactation yield. The animals used were those in which infection was detected within 5 months of the start of the lactation. (Infections may have taken place up to 3 months before they were detected, since this was the interval between tests.) The records of thirty-two animals only were suitable for this analysis.

For each infected animal there was found the gain or loss in yield in the lactation in which infection occurred relative to the yield in the immediately preceding lactation. From this was subtracted the average gain or loss shown by its controls in the corresponding lactation. This gave for each infected animal a single figure representing the apparent gain or loss due to infection. An example of this calculation is shown in Table 3.

Table 3

	gal.
Cow 64 in herd D, infected in fourth lactation:	
Yield in third lactation	834
Yield in fourth lactation	634
Difference	−200 (i.e. loss)
Eight cows in herd D, not infected, and controlling cow 64 in respect of the calving dates of the third and fourth lactations:	
Average yield in third lactation	699
Average yield in fourth lactation	746
Average difference	+47 (i.e. gain)
Hence apparent loss due to infection	247

Again the variability was rather high: from an apparent gain of 84 gal./lactation to an apparent loss of 504 gal./lactation. Apparent losses were found in all but two instances.

There were no significant differences between the average figures obtained for the different herds, or for the different lactation groups. The average figures were as shown in Table 4.

The average apparent loss due to infection for all thirty-two animals was 194·2 gal./lactation, with a standard error of 28·3 ($t = 6{\cdot}86$). The average yields of the control and infected animals in the lactations in question were as shown in Table 5.

This loss has been calculated on the assumption that the infected animals should have shown the same absolute gain in their infected lactation as did the controls in their corresponding lactation. There is no proof, however, that they might not in fact have gained proportionately more or less than the controls. There was indeed some suggestion

in the control group of a negative correlation between the milk yield in one lactation and the gain in the following lactation. On investigation, this correlation was found to be −0·315, which, in the sample of thirty-two cases, was not significant. Even if it had been so, it was calculated that this would have reduced the estimated difference of 194 gal. by no more than about 33 gal.

Table 4

		No. of infected animals	Apparent loss due to infection (gal./lactation)
Herd	A	2	291·0 ± 114·3
	B	7	120·4 ± 61·1
	C	5	193·8 ± 72·3
	D	18	212·2 ± 38·1
Lactation in which infection occurred	Second	14	150·9 ± 43·7
	Third	9	226·3 ± 54·4
	Fourth	6	237·8 ± 66·7
	Fifth	3	212·3 ± 94·3

Table 5

	Controls (gal.)	Infected (gal.)
Preinfection lactation	639·8	743·7
Infection lactation	711·7	621·4
Gain or loss	+71·9	−122·3

Hence apparent loss due to infection is 194·2 gal., or about 24 % of the expected yield of (743·7 + 71·9) = 815·6 gal.

DISCUSSION

The evidence that those animals which became infected with *Strep. agalactiae* produced significantly more milk before infection than did controls in the corresponding period may mean either that there is a direct relationship between inherited ability to produce high yields and susceptibility to infection, or that, by the production of a higher yield than it is normally capable of, the udder suffers some strain which predisposes it to infection. The present data appear to support the former rather than the latter explanation, and are opposed to the findings of Ward in New Zealand (3,4,5), who however used butterfat production as an assessment of yield and employed the cell count method in the diagnosis of mastitis.

Most of the previous work on the effect of bovine mastitis on milk yield has been concerned with mastitis in general and not simply with infection with *Strep. agalactiae*. Frequently clinical mastitis only was considered. The methods of investigation have varied widely, and they are seldom comparable with those used in this study. Minett & Martin (2), in their important paper on the influence of mastitis on milk yield, are concerned mainly with *Strep. agalactiae* and the average effect of the infection, as was done in the present investigation. They compare, however, the yields of healthy and diseased animals in different lactations, and also they include in their analyses the records of animals infected in their first lactation, though they produce no evidence to show that the infected animals were not naturally lower yielders than those that remained healthy. The present work was also concerned with the general overall effect on yield of infection by *Strep. agalactiae* only, and rigorous methods of control were applied in the comparison of infected and non-infected yields. The evidence submitted suggests that in a reasonably well-managed herd infected with *Strep. agalactiae* the average loss in yield in the first lactation of infection may be about 24 %. If the same order of loss were to be carried into later lactations with

infection, then in a herd with the average rate of infection of about 15%, the loss in milk due to *Strep. agalactiae* would be about 3–4% of the total herd yield.

SUMMARY

The records of milk yield and infection with *Streptococcus agalactiae* in four herds over periods of 9–13 years were analysed, using rigorous methods of control in the comparison of yields of infected and healthy cows. Fifty-seven cows which became infected in the second to sixth lactations yielded significantly more ($56{\cdot}5 \pm 20{\cdot}4$ gal./lactation; $P = 0{\cdot}01$) before infection than did non-infected animals in corresponding periods. In thirty-two animals infected in the second to fifth lactations an average apparent loss of $194{\cdot}2 \pm 28{\cdot}3$ gal. was found for the first lactation in which infection was detected. This represents a loss of about 24% of the lactation yield.

This work was initiated by the late Dr A. Cunningham, and was based on the bacteriological data accumulated by him and his collaborators. Grateful acknowledgement is made to Dr N. Lawley for his invaluable help and advice in the statistical analyses, and to Dr T. Gibson for much help in the preparation of the paper. Thanks are due to the Agricultural Research Council for financial assistance.

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448. MACHINE MILKING RATE AND MASTITIS

By F. H. DODD and F. K. NEAVE

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(With 3 Figures)

It is generally accepted that the udder possesses two main defence mechanisms against mastitis infections. The teat sphincter acts as a barrier against the entry of the pathogenic bacteria frequently present on the skin of the teats, and internal factors prevent the bacteria which gain access from setting up an infection. In recent years studies have been made on the relative importance of these two mechanisms, and particular attention has been paid to the relation of teat patency or ease of milking with the incidence of disease. Little (1) stressed the importance of the intact sphincter as a barrier, and suggested that the sphincters of older cows relax, making them more susceptible to infection. In an attempt to overcome the disadvantages of the manual assessment of teat patency, Espe & Cannon (2) used a special instrument. The results were not wholly satisfactory and no correlation with mastitis was found. More recently, Murphy (3) manually examined the teats of at least eighty-seven cows and found a trend towards a higher incidence of infection in quarters with patent teats. This latter author did not agree that relaxing of the teat sphincters with advance in age could account for the increased susceptibility of older cows to streptococcal mastitis (4). While Murnane (5) could not confirm Murphy's results, McEwan & Cooper (6), again using the manual method, obtained further evidence definitely suggesting that easy milking cows were more prone to mastitis. Although Stuart & Lancaster (7) found evidence confirming the importance of the teat orifice, they concluded that it was of less importance than those factors operating within the udder.

The main criticism which can be made of these earlier studies is that, apart from the work of Espe & Cannon (2), they were based on a subjective method of assessment of teat patency. While it is relatively simple to grade the very easy and very hard milking cows in this way, it is very difficult to do this for the majority of cows which fall within these two extremes. The results given below present further evidence of the importance of the teat sphincter, and they are of particular interest since the assessment of ease of milking was by an objective and definite measurement.

SOURCE OF DATA

The results presented in this paper are from studies carried out in the herd of this Institute (herd N), where an intensive mastitis investigation has been in progress since 1945. For details of the frequency and methods of diagnosis, and for the method of assessment of infection, reference should be made to other publications (8, 9). In general, this herd of Shorthorn, Friesian and Guernsey cows, which has been machine milked over the whole period under review, has had little mastitis caused by *Streptococcus agalactiae*, but a relatively high staphyloccoccus (coagulase positive) incidence.

In the same period a study has been made of the rate at which cows milk by machine. The method of measuring milking rate has been to suspend the milking machine on a spring balance during milking and to take balance readings at frequent intervals (10). Details of this study, which has been published elsewhere, has revealed the large differences between the milking rates of cows and the relatively small variations in the milking rates of individual cows at various stages of lactation and with advance in age (11).

In order to study the factors, milking rate and mastitis, the milking-rate records of all cows in the herd have been collected at three successive morning milkings in about the fifth week of lactation. Since it has recently been found that the rate of machine milking is governed by the vacuum and the effective size of the teat orifice, these records are an objective measure of teat patency (12).

RESULTS

Although our results do not confirm Little's suggestion that teat patency increases with advance in age, the data presented here is from first lactations only. This has been done because of the known differences in susceptibility of animals of different ages, and because in the herd under review there was an involuntary selection for faster milking. While culling was not very severe and was mainly carried out on the basis of milk yields, it appears that the slower milking cows tended to be culled at an earlier age. To facilitate comparison the 'peak flow' has been taken as a measure of the milking rate of each cow. This is the maximum amount of milk which flows from the cow during any one minute of milking. The peak flow records of the ninety-four heifers used in this study varied from 1·0 to 7·4 lb./min., and the animals have been classified into five speed groups as shown in Table 1.

Table 1. *The classification of ninety-four first-lactation cows into five groups according to their milking rates (peak flow) in early lactation*

Group	Description	No. of heifers	Mean peak flow of group (lb./min.)
1	Peak flow under 3·0 lb/min.	20	2·42
2	Peak flow between 3·1 and 4·0 lb./min.	15	3·56
3	Peak flow between 4·1 and 5·0 lb./min.	26	4·50
4	Peak flow between 5·1 and 6·0 lb./min.	24	5·55
5	Peak flow over 6·1 lb./min.	9	6·79
		Total 94	

The total number of types of the new infections which occurred in each group is shown in Table 2, which also shows the number and types of the new infections starting in the first dry period. In Table 3 the number and proportion of cows in each group which became infected and clinical are given, and because staphylococcus infections were the most important, data for the number of cows infected with this organism are also presented. The results given in these tables are also shown graphically in Figs. 1–3, and need very little interpretation. Clearly, with increasing milking rate, there is a marked and progressive increase in the amount of mastitis occurring in the first lactations. This applies to subclinical and clinical mastitis in the lactation and also to the incidence of dry-period infections.

It is interesting to record that of these ninety-four heifers six were infected at their first calving, four of these had *Strep. uberis* infections, one *Strep. dysgalactiae* and one a coliform infection. Four of these six cows had milking rates of over 5·1 lb./min.

Table 2. *The amount and type of infection occurring in the first lactations of the ninety-four heifers grouped according to rate of milking*

			Types of new infections							Types of clinical infections							Types of new dry-period infections					
Groups	Nos. of cows	No. of cows infected	*Staphylococcus*	*Strep. agalactiae*	*Strep. dysgalactiae*	*Strep. uberis*	Coliform	Total	No. of cows clinical	*Staphylococcus*	*Strep. agalactiae*	*Strep. dysgalactiae*	*Strep. uberis*	Coliform	Total	Cows infected in first dry period	*Staphylococcus*	*Strep. agalactiae*	*Strep. dysgalactiae*	*Strep. uberis*	Coliform	Total
1	20	2	1	—	—	2	—	3	1	—	—	—	1	—	1	3	2	—	—	3	—	5
2	15	6	5	1	1	—	—	7	3	1	1	1	—	—	3	2	1	—	1	—	1	3
3	26	12	12	1	—	1	—	14	5	5	—	—	—	—	5	6	4	—	—	2	1	7
4	24	10	15	6	1	—	—	22	10	11	4	—	—	—	15	8	6	—	1	4	2	13
5	9	6	6	3	1	—	—	10	4	3	3	1	—	—	7	4	5	—	—	5	—	10
Totals	94	36	39	11	3	3	0	56	23	20	8	2	1	0	31	23	18	0	2	14	4	38

Table 3. *The proportion of cows becoming infected and the number of infections per cow*

Group	Proportion of cows becoming infected (%)	Proportion of cows becoming clinical (%)	Proportion of cows becoming infected in dry period (%)	No. of new infections per cow	No. of new staphylococcal infections per cow	No. of clinical infections per cow	No. of new dry-period infections per cow
1	10·0	5·0	15·0	0·15	0·05	0·05	0·25
2	40·0	20·0	13·3	0·47	0·33	0·20	0·20
3	46·2	19·2	23·1	0·54	0·46	0·19	0·27
4	41·7	41·7	33·3	0·92	0·63	0·63	0·54
5	66·7	44·4	44·4	1·11	0·67	0·78	1·11

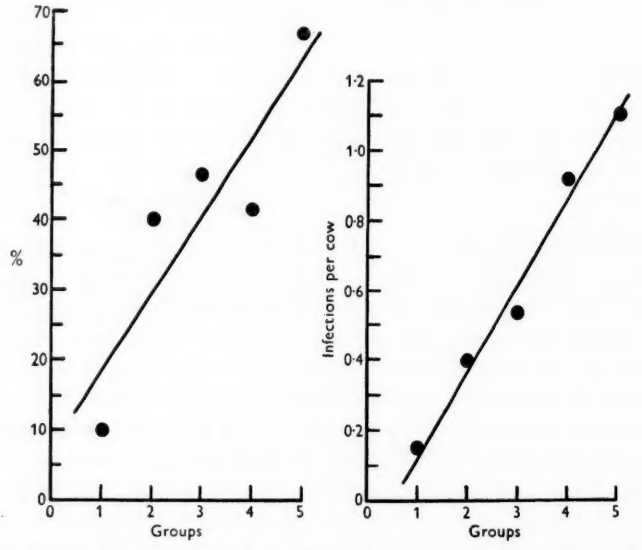

Fig. 1. The proportion of cows becoming infected, and the mean number of infections per cow.

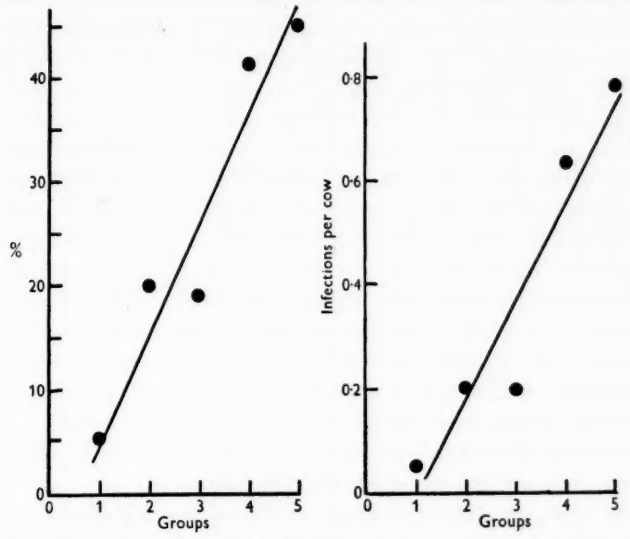

Fig. 2. The proportion of cows clinically affected in first lactations, and the mean number of clinical outbreaks per cow.

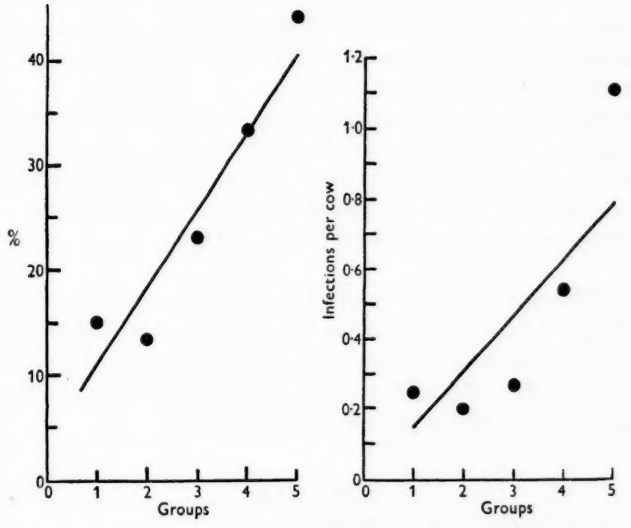

Fig. 3. The proportion of cows becoming infected in first dry period, and the mean number of new dry-period infections per cow.

DISCUSSION

The experimental data given in this paper provides, we believe, good evidence that with first-lactation animals there is a strong correlation between milking rate and the incidence of clinical and subclinical mastitis. Although Stuart & Lancaster (7) have suggested that the teat barrier is of less importance than factors operating within the udder, we feel that this is not yet proved since the data was based on quarters which became re-infected and, as Stuart & Lancaster themselves pointed out, 'it is possible that the primary infections caused changes within the udder which influenced the susceptibility to infection by infusion'. Until further research is carried out, it will be difficult to assess the relative importance of these factors and of their importance in herd control. Differences in milking rates cannot account for the large differences in mastitis incidence rates which are found between separate herds, although it is probable that severe culling of slow-milking cows would be followed by an increased mastitis problem. Within a herd it will account for some of the differences in susceptibility between cows and, in part, for the increased incidence in older cows if the slow-milking cows are culled at an early age. Further evidence on this and the correlation in older cows will be given in a later publication.

It is interesting that this is an example of the way in which heredity affects the susceptibility to disease, since milking rate is an inherited character.

Although the most probable explanation of the increased mastitis incidence of fast-milking cows is that bacteria can more readily gain entrance to their udders through larger or slacker teat sphincters, there is another possible explanation. Dodd *et al.* (13) have provided some evidence that leaving milking machines on the teats longer than is necessary to remove the milk is likely to increase infection, and it is reasonable to suppose that, to some degree, this is more likely to occur with the faster milkers. However, we feel that this factor is of minor importance since, by the same reasoning, we would expect the front quarters to have a higher incidence than hind quarters, which is contrary to most of the published evidence (14, 15, 16, 17, 18).

McEwan & Cooper (6) recommended the breeding of slow-milking cows as a possible way of controlling mastitis. We cannot agree with this proposal for, apart from the tedium and high labour cost of milking slow-milking cows, we feel that effective methods of mastitis control can be devised by less costly means, since it is not uncommon to find herds containing a proportion of fast milkers with a low incidence.

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449. THE DETERIORATION OF MILKING RUBBERS

I. THE EFFECT OF MICRO-ORGANISMS

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It has been shown by a number of investigators that rubber is subject to microbial attack. In particular, the work of Söhngen & Fol (1), Spence & van Niel (2), Spence & Ferry (3) and ZoBell & Grant (4) may be cited. Although ZoBell & Beckwith (5) have shown that vulcanized rubber is less readily attacked than raw rubber, the question of the importance of the microbial degradation of the rubber of the teat-cup liners of milking machines arose. This rubber forms a special habitat inasmuch as it contains, during use, a steadily increasing proportion of fat; it is frequently wet with milk, and after washing it often remains wet for some time. It is not on all farms that liners are frequently steamed, and even when steaming is practised the interior of the rubber will be relatively dry, and dry organisms are resistant to heat. It seemed not impossible, therefore, for micro-organisms to grow in a layer of fat, or to be carried by fat into crevices or pores in the rubber where a sufficient number might be protected by dehydration to form fresh growth during the periods between steamings. Indeed it was confirmed that the greasy rubbery mixture which may be scraped from old liners, and the small pores which sometimes occur in the less well-made rubbers, have a rich flora.

The presence of sulphur in vulcanized rubber suggested that micro-organisms which oxidize sulphur might be able to soften rubber. Thaysen *et al.* (6) have found, for example, that sulphur bacteria growing in rubber hose produced enough sulphuric acid to rot the canvas. Although liners, being greasy, impose different conditions, it was felt desirable to ascertain whether degradation by sulphur bacteria could be excluded from consideration as a cause of deterioration.

Although some interesting results were obtained, all the findings were, from the practical point of view, negative. For this reason they are reported only briefly.

METHODS

Obtaining inocula. At first pieces of the softest parts of old liners were rubbed aseptically in sterile olive oil or butter fat with a glass rod, but it was impossible to obtain fine subdivision, so that later the more convenient technique of shaking with glass beads in Ringer's solution was used and found to be adequate. Many of the liners used were particularly greasy, and had been discarded for some time. By scraping hard with a blunt sterilized knife it was easy to remove from their surface a few milligrammes of the most degraded rubber, mixed with a proportion of fat. This soft mixture emulsified sufficiently in the Ringer's solution to give an inoculum. In some of the liners the inner surface showed a number of swollen areas, with an increase in thickness of, perhaps, a millimetre with occasionally a few white fibres like hyphae upon them. Inocula were also taken from these.

Media for testing degradation. For testing the effect on a continuous film of rubber, fat and fillers, the following mixture was used. Pale crepe (5 g.), and 3 g. butter fat, were

dissolved in 200 ml. benzene. To the solution was added 2 g. chalk and 2 g. charcoal, both finely powdered. After mixing, 3 ml. quantities were roughly measured into sterile test-tubes and dried as slopes at about 70°. To these was added aseptically a suitable sterile mineral solution, e.g. 1 g. NaCl, 1 g. $MgSO_4$ $7H_2O$, 0·05 g. KNO_3, 1 g. K_2HPO_4, 1 g. citric acid in 1 l. of hard tap water finally adjusted to pH 6·9 with NaOH. On some occasions a soil extract was used. Enough solution was added to cover half the rubber film. In some experiments Lemco broth was added to the rubber film. Rubber films without fat or without fillers were resistant.

For testing the effect of cultures on pure rubber alone the latex agar medium of Spence & van Niel(2) was used. Ammonia-preserved latex was diluted several-fold with approximately 0·05 M-potassium phosphate buffer at pH 6·8 and dialysed against the same buffer until free from ammonia. The latex could then be sterilized by autoclaving with but little coagulation. Dilution to approximately 1% of rubber with a sterile mineral medium containing 0·1% $(NH_4)_2SO_4$, 0·1% KH_2PO_4 and 0·05% $MgSO_4$ $7H_2O$ at pH 7 gave a suitable liquid medium. The solid medium was, however, more useful. For this the mineral solution containing agar was first poured, and after it had solidified a thin layer of 1% agar containing just enough latex to produce a definite milkiness was allowed to solidify over the mineral agar. On this medium certain streptomyces form beautiful clear zones after a few days' incubation.

Estimation of sulphur bacteria. All the sulphur organisms likely to be present decompose sodium thiosulphate, and the media used by Starkey(7, 8) were therefore chosen, of which the following will serve as an example:

Tap water	1000	$MgSO_4$ $7H_2O$	0·1
$Na_2S_2O_3$ $5H_2O$	10·0	$MnSO_4$	0·02
$(NH_4)_2SO_4$	0·1	$FeSO_4$	0·02
K_2HPO_4	2·0	pH adjusted to	7·8
$CaCl_2$	0·1		

As in this case the water was particularly hard it was impossible to avoid some precipitation on sterilizing. This added to the difficulty of observing the organisms in liquid culture. Titrations were therefore used and comparisons made between the decomposition of thiosulphate in the cultures and that in the sterile medium under the same conditions. The suitability of the medium was demonstrated by the ease with which sulphur bacteria from soil grew in it. On the same medium solidified with agar, macroscopic colonies were formed.

RESULTS

Organisms growing in rubber and fat

In a number of early experiments breakdown of a rubber + chalk + charcoal film covered with the mineral solution in Petri dishes occurred several weeks after inoculation with dilutions in oil of the oily inocula already described. Numerous subcultures prepared from such degraded films were strongly lipolytic, but were not able to attack films of pure rubber.

Repeated experiments of this kind, with variations, failed to demonstrate adequately any degradation of films consisting of rubber only. The final experiment is described in more detail.

Oily inocula from washed liners were added to test-tubes of the rubber + fat + chalk + charcoal medium partly covered with soil extract. After 3 weeks' incubation at 26° growth

in the form of small opaque masses floating in the liquid or resting on the bottom of the slopes was observed. After a further 3 weeks cultures representative of all the rubber samples showed erosion at the base of the tubes where the growth had been resting on the rubber.

Inoculation from these tubes into a fresh medium containing no fat showed that erosion did not take place in the absence of fat.

Further cultural experiments showed that in only one of the cultures was there an organism capable of digesting rubber in the latex agar medium. This had the characteristics of a streptomyces. The other organisms appeared to be cocci, and it seems probable that the breaking up of the fatty rubber films in the first part of the experiment was due to the action of lipolytic organisms in producing fatty acids and soaps, thus rendering parts of the film soluble, parts brittle. This confirms earlier experiments in which the disintegration of rubber-fat films was observed.

In other attempts to isolate rubber-digesting organisms from liners, disintegrated scrapings were plated on the latex agar. Liners from three out of five farms gave, after several weeks, colonies surrounded by clear zones. The smell, texture and microscopic appearance of the colonies suggested that they were all streptomyces. Streptomyces capable of digesting rubber occur in soil, and their presence on liners is possibly the result of soil contamination rather than actual growth on the rubber. Therefore the numbers of rubber-digesting streptomyces and the numbers of other streptomyces were determined for three liners and for three samples of soil.

The results are shown in Table 1, from which it is clear that there was not a significant preponderance of rubber-digesting micro-organisms in the liners.

Table 1

	Liners			Soils		
Source of inoculum	A	B	C	1	2	3
Total no. of streptomyces colonies on latex agar plate	8	152	5	462	642	41
No. of rubber-digesting colonies	5	2	1	22	42	1

Pure cultures of several of these streptomyces were used for experiments on their nutrition and enzyme production, with the following results:

(1) Previous culture on latex reduced the time required for the formation of zones of digestion from 12 days to about 3 days.

(2) The presence of plentiful easily assimilable nutrients (yeast dextrose agar) prevented the digestion of the rubber.

(3) Small quantities of easily assimilable nutrients delayed the digestion of the rubber.

(4) Peptone and yeastrel added to latex agar diminished the digestion of the rubber.

(5) Ammonium sulphate stimulated the digestion of the rubber.

(6) The mixture of salts recommended by van Niel (see Methods) was slightly superior to soil extract for demonstrating rubber digestion.

(7) The power to form clear zones in latex agar was not lost when the streptomyces were cultivated for several generations in yeast dextrose agar.

A number of attempts to prepare the enzyme were made. One of these consisted in growing the organism on broken glass in a 5 l. vessel which was alternately filled with medium and emptied. It was not possible to demonstrate the presence of the enzyme in the cell-free liquid, although digestion in liquid cultures had occurred.

The experiments described above had already shown that the microbiological degrada-

tion of rubber is a slow process. To confirm that it is not of practical importance in the deterioration of liners, the effect on the liners themselves of the most vigorous rubber digesting streptomyces available was tested. Two liners of the stretched type, one old, one new, were cut into two at right angles to their lengths and each portion was converted into a closed tube by stoppering one end with a rubber bung and plugging the other with cotton-wool. After half filling the four tubes with the mineral solution used for the latex agar and sterilizing them, one of each pair was inoculated with the streptomyces. The tubes were inspected after $2\frac{1}{2}$ and 4 months at 26°. The second time they were dry. They were therefore split open, and a comparison of the inoculated rubbers with the controls showed that the streptomyces had produced slight marks on the surface of the new liner, and in the old one had formed more swollen areas like those occasionally to be seen in discarded liners (see Methods; Obtaining inocula).

It is clear therefore that attack of the rubber does occur, but it is far too slow to be of practical importance under temperate conditions. The existence of numerous lipolytic organisms in the absorbed fat is likely to be of greater importance as a possible source of taint or contamination from ill-washed liners.

Sulphur bacteria

Some interesting cultures on thiosulphate agar were obtained from liners, some producing precipitates, some granules. Microscopic examination showed, in the different cultures, a variety of forms one of which was motile.

Scrapings from old liners were dispersed in Ringer's solution as for the isolation of streptomyces, and the suspension used to inoculate 25 ml. quantities of the medium. Similar inoculations were made with the dust of the shelves or dairies where the liners had

Table 2. *Bacterial decomposition of thiosulphate*

Source of culture	Thiosulphate decomposed in 12 days (%)	Thiosulphate decomposed in 22 days (%)
Linear no. 1 (inside)	32	45
Linear no. 1 (outside)	8	26
Dust no. 1	26	53
Linear no. 2 (inside)	7	46
Linear no. 2 (outside)	28	2
Linear no. 3 (inside)	0	0
Linear no. 3 (outside)	0	0
Linear no. 4 (inside)	0	28
Linear no. 4 (outside)	0	0
Dust no. 4	0	1·2
Linear no. 5 (inside)	0	0
Linear no. 5 (outside)	0	9·4
Dust no. 5	16	60

been lying. Samples of the medium were titrated after 12 and 22 days. In Table 2 the concentrations of thiosulphate decomposed are expressed as percentages of the concentration in the blank at the appropriate time. (The decrease in concentration of the blank is of the order of 8% in 3–6 weeks.)

It is clear that on the whole liners are not a richer source of sulphur bacteria than the dust of their surroundings. Thus these organisms do not multiply upon the liners. This was confirmed by inoculating a mineral medium without thiosulphate in sterilized liners with sulphur bacteria. After several months' incubation, there was no effect upon the rubber.

DISCUSSION

It is interesting to have confirmed the commonness of micro-organisms capable of decomposing rubber, and of the sulphur bacteria, and to have demonstrated the ease with which they may be isolated once suitable media have been made. Under certain conditions, perhaps in the tropics, such micro-organisms may contribute to the deterioration of rubber goods, but under farming conditions in this country they are not likely to affect the life of the rubber parts of milking machines.

SUMMARY

It has been shown that, although they are present, sulphur bacteria and streptomyces able to oxidize rubber do not affect the life of the rubber teat-cup liners of milking machines.

The author thanks the Deputy Director, Dr A. T. R. Mattick, for his encouragement, the Avon India Rubber Company for their help and Mrs Pamela Waterhouse for technical asistance.

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450. THE EFFECT OF THE PRESENCE OF AIR ON THE HEATING OF FULL MILK BOTTLES BY STEAM

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(With 4 Figures)

Heating by means of saturated steam is used widely in the dairy industry in many processes. One application is autoclaving, a process not confined to the laboratory but used also for the preparation of sterilized milk (1). It is well recognized that the presence of air in autoclaves depresses the temperature of the heating medium when control of the process is based on measurement of pressure, but it does not appear to be generally appreciated, at least in the dairy industry, that air can depress the rate of heating of the load even when thermostatic devices are used to control the autoclave temperature. In the first place air will tend to accumulate in areas remote from the steam inlet in large vessels, with consequent reduction in partial steam pressure, and hence steam temperature, in these areas. Where the atmosphere temperature is maintained another effect occurs. This second effect is due to insulating layers of air which form on the cool surfaces of the load as the steam fraction of the mixed atmosphere condenses. A blanket of air thus formed is unable to disperse because of the general movement of the mixed atmosphere to the condensing surface.

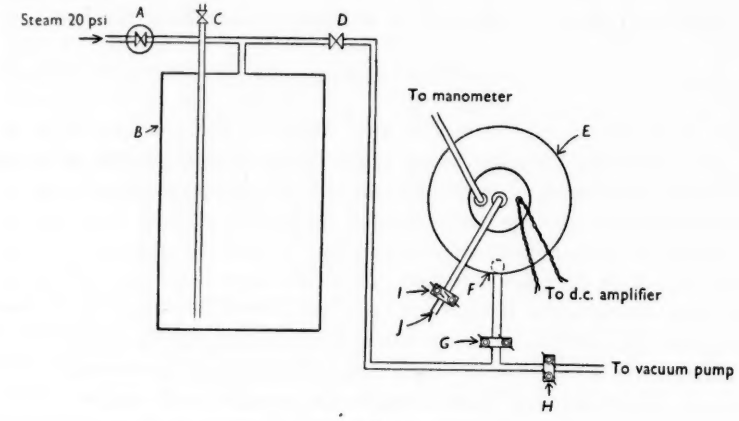
The ability of blankets of non-condensable gases to reduce the effectiveness of heat transfer is well recognized in other industrial fields. For example, its effect on the performance of large steam condensers is discussed by Haywood (2), and a more general appreciation is given by Lyle (3). Quantitative data showing the effect of air on heat transfer in relatively quiescent atmospheres such as are found in autoclaves have, however, not been published. The following work was undertaken to assess the importance of the effect when filled milk bottles are heated by means of saturated steam, the general temperature of which is constant.

EXPERIMENTAL

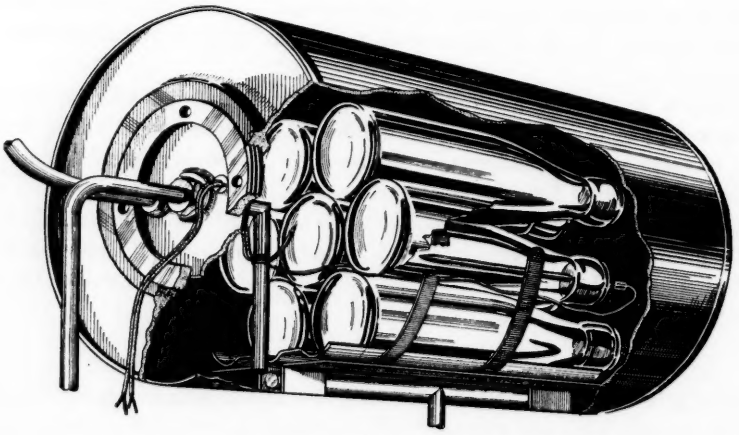
Seven 1-pint glass milk bottles filled with water and closed with bungs wired down were arranged in contact with one another inside a cylindrical vessel *E* as shown in Fig. 1. Temperature readings were taken at about the middle of the centre bottle by means of a 34-gauge copper-constantan thermocouple supported by a closed glass tube passing through the rubber bung. The last 2 cm. of the glass tube were drawn down to about 2 mm. and filled with water to increase heat transfer to the junction.

Steam from a low-pressure main (20 psi) was admitted by valve *A* (Fig. 1), equipped with a large hand wheel, to a surge tank *B* of about 3·5 cu.ft. capacity with a condensate outlet *C*. By means of valve *D* steam could be admitted underneath a false bottom in vessel *E*, through the horizontal header *F* which was perforated on the underside. By means of screw-clips *G* and *H* on the hose connexions the vessel could be evacuated or the

connexions filled with steam. The lid of the vessel carried the condensate outlet *I*, controlled by screw-clip *J*, and a connexion to a mercury manometer. Two pairs of thermocouple wires also passed through the lid, one pair from the junction in the experimental bottle, and the other from a junction which was used to measure the temperature of the atmosphere in front of the experimental bottle. The output from either thermocouple was selected by a manually operated switch and recorded by means of a d.c. amplifier in conjunction with a recorder.



(*a*)



(*b*)

Fig. 1. Experimental equipment. *a*, diagram of layout; *b*, arrangement of bottles in heating vessels.

After evacuating the vessel to a suitable pressure, due allowance being made for water-vapour tension, steam was admitted until the pressure increased by 30 in. Hg. This pressure was then maintained constant within $\pm 0{\cdot}1$ in. by means of valve *A*. When complete elimination of air from the vessel was required the vessel was first evacuated to a residual pressure of less than 1 in., steam admitted to warm the water lying in the bottom of the vessel, and evacuation then continued, the water boiling freely and eventually cooling to the original temperature. It was assumed that this treatment eliminated residual air.

Partial pressures of air of 0, 2½, 5, 15 and 30 in. Hg were used in conjunction with a constant pressure of 30 in. Hg of saturated steam. In one experiment the vessel was operated throughout at atmospheric pressure: steam was admitted through the usual orifice, and air was displaced through the condensate outlet which had been turned to point upwards.

RESULTS AND DISCUSSION

The rise in temperature with time of the water at the centre of the bottle for various partial pressures of air is shown in Fig. 2. Also shown is a curve showing the rise in temperature at the same point when steam was allowed to displace the air initially present at atmospheric pressure. Each curve represents the average of two tests, the differences between each two curves being small.

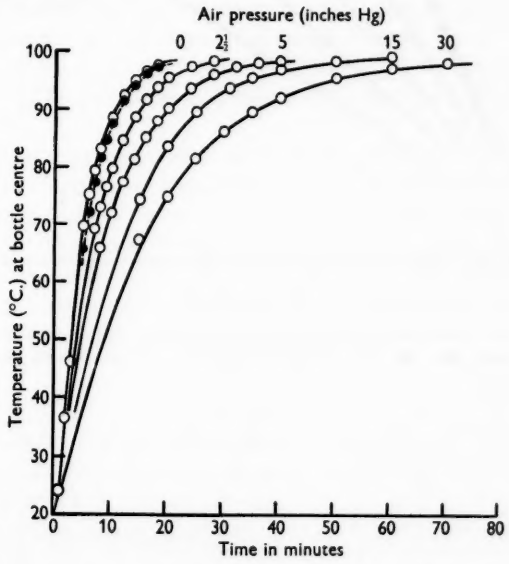


Fig. 2. Heating curves of various partial pressures of air in steam at 100° C. ●--●, steam allowed to displace air at atmospheric pressure.

The air-steam atmosphere rose to its maximum temperature after 1–1½ min. in every case. It was found that, under the conditions of the experiment, the addition of 1 atmosphere of steam to the air initially present did not always give an atmosphere temperature of 100° C. The temperature was consistent for different tests at the same air content, but varied with air pressure between 96° C. at 30 in. Hg and 100·5° C. at 2½ in. Hg. The bottle temperature appeared to be asymptotic to the actual atmosphere temperature, although in some cases the bottle temperature after a considerable heating period actually exceeded slightly the measured atmosphere temperature. The fall in atmosphere temperature with increasing air pressure was probably due to the accumulation of air in excess at different parts of the vessel, causing variations in the partial pressure of steam and hence in the atmosphere temperature.

To enable a better comparison of the curves to be made, small corrections were applied to make the curve for each air pressure asymptotic to 100° C.

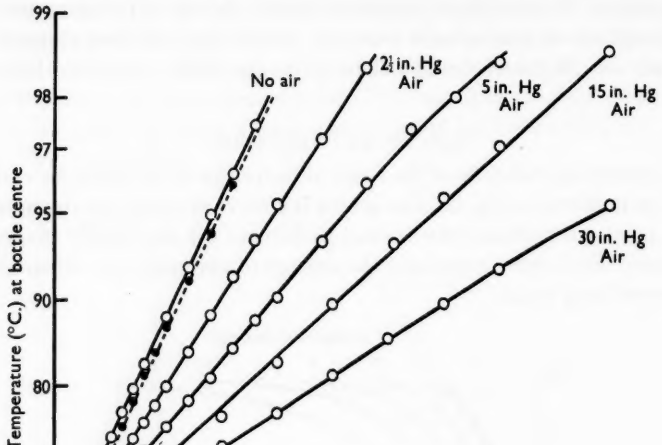


Fig. 3. Heating curves for various partial pressures of air in steam at 100° C. (with logarithmic temperature co-ordinates). ●--●, steam allowed to displace air at atmospheric pressure.

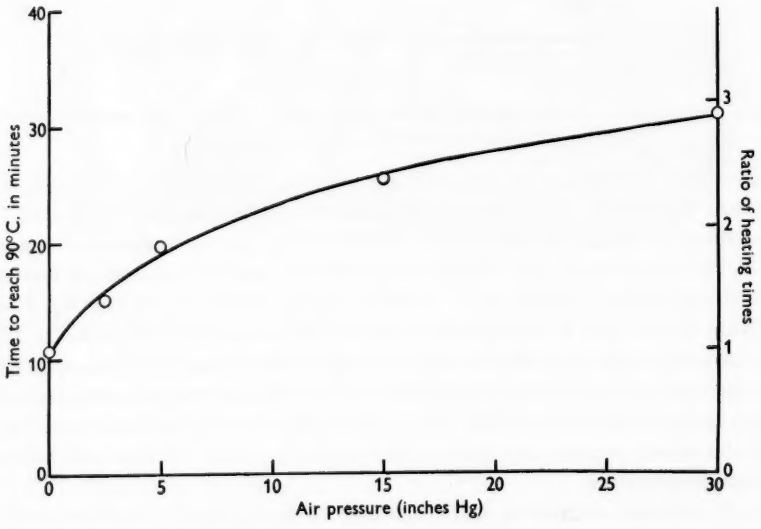


Fig. 4. Variation of heating time with partial pressure of air in steam at 100° C.

It can be seen that the partial pressure of air has a considerable effect on the heat transfer from the steam to the bottle contents. For comparison of the rates of heating it is convenient to re-draw the heating curves, using a logarithmic temperature scale (Fig. 3). If the rise in temperature follows an exponential curve, the transformed curves should be linear. This is the case except for a very slight curvature at high temperatures which appears in some of the curves.

The resistance to the transfer of heat from the steam to the liquid at the centre of the bottle is inversely proportional to the slope of the lines. The effective thermal resistance can therefore be represented by the time required to reach any selected temperature from an initial temperature of 20° C. The time required to reach 90° C. is shown as a function of the partial pressure of air in Fig. 4. The ratio of the time at any air pressure to the time required in the absence of air is shown on the subsidiary scale. These ratios apply to the time to reach any temperature in the exponential section of the curves, and therefore apply at temperatures between 60 and 98° C. approximately.

It can be seen that the heating times increase rather quickly for partial pressures of air below about 5 in. Hg. They continue to increase with larger amounts of air, but to a somewhat reduced extent.

With the chamber open to atmosphere, and steam displacing the air, the rise in temperature is given by the broken line in Figs. 2 and 3. There is a slight initial lag in the temperature rise, but as the heating continues the curve becomes more nearly coincident with that in the absence of air. These results are consistent with the removal by the flow of steam of an air layer, present in the early stages of heating, from the surface of the bottle. The air is removed sufficiently rapidly for there to be no appreciable difference between the heating curves in the initial presence or absence of air.

CONCLUSIONS

1. The rate of heating of liquid-filled bottles by steam at 100° C. is greatly affected by the presence of air. The rate of heating decreases steadily with increasing air pressure.
2. Under the conditions of the test air was easily displaced by steam when the heating chamber was open to the atmosphere, and heating then proceeded as rapidly as in the initial absence of air.

We are indebted to C. Machin for preparing the figures for publication.

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451. BIOCHEMICAL AND SEROLOGICAL PROPERTIES OF SOME CITRIC ACID FERMENTING STREPTOCOCCI FROM MILK AND DAIRY PRODUCTS

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INTRODUCTION

Several investigators (1, 13, 14, 15, 22, 33, 34) have isolated, mostly from dairy starters or dairy products, strains of streptococci which are able to produce relatively large amounts of lactic acid in milk as well as acetic acid, carbon dioxide, diacetyl and acetoin. These streptococci are of considerable importance in butter starters (23, 33, 34) and in the ripening of certain types of cheese (34, 36).

In some cases it has been shown that the lactic acid formed is dextrorotatory (1, 15, 23, 34), and that the production of gas and C_4-compounds results from the utilization of citric acid (2, 33, 34). These strains obviously differ essentially from the Leuconostocs. In respect of sugar fermentations they resemble the lactis-cremoris streptococci (22, 33, 35), but the breakdown of citric acid suggests a relationship with the enterococci (6, 35).

The aim of this work is to define biochemically a number of citric acid fermenting strains by means of those reactions which have proved useful in the classification of the enterococci and of the lactis-cremoris group. Since the enterococci belong to serological group D, and *Streptococcus lactis* and probably *Strep. cremoris* belong to group N, it was likely that a serological investigation would determine the identity of the citric acid fermenting strains under investigation.

MATERIAL AND METHODS

Bacterial strains

Most of the strains used for this investigation were isolated from starter cultures or raw milk in Sweden. Freshly coagulated starter cultures or spontaneously soured milk was plated on a medium containing peptone 0·5%, meat extract (Difco) 0·3%, lactose 0·2%, K_2HPO_4 0·5%, $MgSO_4$, $2H_2O$, 0·4%, tri-sodium citrate 0·2%, yeast autolysate (1%N)

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1% and agar. Single colonies were transferred to litmus milk. Those which clotted the milk and reduced litmus in 24 hr. were transferred to plain milk and tested for the production of acetoin by means of the Voges-Proskauer reaction. The positives were again plated, and well-isolated colonies picked and again re-tested in the same way.

The strains thus isolated were 52–57, isolated from a starter culture in practical use, 100–114, isolated from raw milk, A2 and A9, isolated from a dairy starter, and B1, B3 and B5, isolated from a dairy starter.

Strains nos. C26/40 and C27/6 were kindly supplied by Miss E. R. Hiscox, who had isolated them from experimental English Cheddar cheese.

Strains nos. 23 C5, 30 C1 and 33 C1 were isolated and investigated by Miss E. I. Garvie from samples of raw milk, from farms in the neighbourhood of Shinfield, at the time of clot-on-boiling. Some strains of *Strep. cremoris* and *Strep. lactis* were included in the investigation for comparison. These strains were obtained from the type collection of the N.I.R.D. Three strains of *Strep. citrophilus* van Beynum & Pette (1), were received from Dr Pette.

Cultural and biochemical methods

Morphology and staining properties were determined in a 24 hr. old milk culture (30° C.). Dowson's (8) method of Gram-staining was used.

Behaviour in litmus milk at 30° C., with special regard to reducing power, was observed.

Haemolysis. Brown's (4) technique was followed, using nutrient agar and horse blood.

Growth temperature range. Two loopfuls (4 mm. diameter) of a 18–20 hr. old culture were transferred to the test media. These were yeast-dextrose litmus milk (0·3% Yeastrel, a commercial yeast autolysate, 1% dextrose, and litmus as indicator in separated milk), and a broth (D.L.B.) containing 1% peptone (Evans), 1% beef extract (Lab. Lemco), 1% dextrose, 0·5% NaCl, tap water, with pH = 7·0. The tubes were incubated in an accurate thermostatically controlled water-bath and growth observed after 6 days (10° C.) and 24 hr. (40 and 45° C.) respectively.

Growth in the presence of sodium chloride at 30° C. 4 and 6·5% NaCl was added to a basal medium containing 0·5% lactose, 0·5% tryptone (Difco) and 0·5% beef extract (Lemco) at pH 7·2.

The tubes containing 5 ml. were inoculated with a loopful (4 mm.) of an 18 hr. culture in D.L.B. of the relevant strain. Control tubes without sodium chloride were also used.

Growth at pH 9·6. The test described by Shattock & Hirsch (29) was used.

Survival at 60° C. *for* 30 *min.* Two loopfuls (4 mm.) of an 18 hr. culture (D.L.B.) were transferred to D.L.B., held at 60° C. for 30 min. in a thermostatically controlled water-bath. The time was reckoned from the moment when the broth in a control tube fitted with a thermometer had reached 60° C. After 30 min. exposure the tubes were cooled in tap water, incubated at 30° C. for 24 hr. and examined for growth.

A second survival test described by Demeter was carried out (7), using milk fortified with Yeastrel.

'Carbohydrate' fermentations. Peptone water (1% peptone, 0·5% NaCl) containing 0·5% of the test sugars, added after sterilization, with litmus as indicator, was inoculated with 1 loopful (4 mm.) of an 18 hr. broth culture and incubated at 30° C. Results were read after 1, 3 and 7 days. Acid production within 3 days is indicated by + and acid production after 7 days by (+).

Hydrolysis of aesculin was shown with $FeCl_3$ solution.

Hydrolysis of arginine. The method of Niven, Smiley & Sherman (20) was used with 0·25% yeastrel instead of the yeast extract in the original medium.

Gelatin liquefaction. Nutrient gelatin was inoculated with a loopful of an 18 hr. old broth culture, incubated at 30° C. for 48 hr. and then observed for liquefaction after cooling for 6 hr.

Production of CO_2

The method of Gibson & Abdel-Malek (9) was followed, using the milk-yeast-tomato medium. Some strains were also tested in plain milk. In these cases CO_2-free nitrogen was passed through a 48 hr. old milk culture, and the liberated carbon dioxide absorbed in a U-tube containing 'Ascarite' (NaOH absorbed on asbestos) after having been dried in a similar tube containing 'Dehydrite' (water-free magnesium perchlorate). The amount of CO_2 formed was estimated by weighing.

Production of acetoin

A basal medium containing 1% tryptone (Difco), 1% beef extract (Lemco), 0·5% yeast extract (Lemco), 0·2% NaCl, 0·5% K_2HPO_4, 0·05% $MgSO_4$, 0·05% $MnSO_4$ was steamed for half an hour, and divided into two portions. To one to these was added 1% lactose, to the other 1% lactose and 0·5% tri-sodium citrate. The media were distributed in 4 ml. quantities in test-tubes 24 mm. wide and sterilized.

After inoculation (1 loopful of an 18 hr. culture) the tubes were sloped to provide good aeration and incubated at 30° C. The Voges-Proskauer reaction was used to detect acetoin formation, and some strains were tested for diacetyl formation by den Herder's (11) method.

Breakdown of citric acid

The method of Sjöström & Emilsson (32) was used for estimating the citric acid in milk or milk with yeast autolysate.

Serological methods

Preparation of anti-sera

The cultures chosen for immunizing rabbits were inoculated into yeast-dextrose-litmus milk and after a short incubation at 30° C., as described by Nichols & Hoyle (19), were stored in a refrigerator. For preparation of the suspensions D.L.B. was inoculated from these stock cultures and incubated at 30° C. for 24 hr. The organisms were removed by centrifuging and resuspended in 0·85% sodium chloride solution.

Two kinds of suspensions were used: (*a*) heat-killed organisms, the suspension was heated to 56° C. for 1 hr.; (*b*) living organisms: a saline suspension was used as a vaccine.

The heat-killed organisms were inoculated twice a week with 1 ml. of a suspension containing the growth from 10 ml. broth in 3 ml. saline (giving an opacity equivalent to Brown's tube no. 10, Burroughs Wellcome and Co., Red Lion Square, London, W.C.1). With this suspension a potent strain-specific serum was obtained, in most cases, after three to six inoculations. When type reactions became stable, as shown by test bleedings, the course of inoculations was altered and living organisms were used. The rabbits were then inoculated every second day for a week, followed by a week's rest. The doses given were, consecutively, 1 ml. of a suspension of the growth from 10 ml. broth in 3 ml. saline, 1 ml. of a suspension of the growth from 20 ml. broth in 3 ml. saline, and 1 ml. of a suspension of the growth from 50 ml. broth diluted to 3 ml. as above (this corresponds roughly

to 1 ml. suspension of an opacity equalling 1, 2 and 6 times Brown's tube no. 10, respectively).

The use of living organisms as vaccine is generally regarded as the best way to obtain good sera, but is unfortunately not always possible. Working with streptococci of groups D and N, Shattock & Mattick (28) sustained some losses of rabbits when using living organisms, and concluded that at least some strains of these organisms have a certain pathogenicity for the rabbit when introduced intravenously. In this investigation, however, only one death was sustained (culture 106) among eleven rabbits. Possibly this fortunate result was due to the fact that the living organisms were not injected until the rabbits had developed strain-specific antibodies.

Extracts

Lancefield's (17) method was used.

Precipitin test

Ring tests were made using the technique described by Shattock (26). All tests were read after 5–10 min. and the negatives again after 30 min.

The technique of Lancefield (17), using dilutions of extract and incubation at 37° C. for 2 hr. followed by standing in a refrigerator overnight, was used for reciprocal absorption tests. (For exceptions see p. 263.)

Absorption test

The organisms used for absorption were grown in D.L.B. for 24 hr., and absorptions carried out as described by Shattock (26).

Typing by agglutinin test

The technique described by Colebrook (5) was followed.

RESULTS

Cultural and biochemical reactions

The strains included in this investigation grow in milk as diplococci or as short chains, but *Strep. citrophilus* strains showed very long chains. All the strains are Gram positive and catalase negative.

The results of the biochemical and cultural tests are collected in Table 1. For the sake of brevity only those strains that have different origins or behave differently in one way or another are included. The second column in the table shows the number of strains investigated, which have properties identical or almost identical with those of the strains mentioned in the table. These 'similar strains' sometimes differ slightly from the typical strains in their behaviour on blood agar. Some show no action and some slight greening.

All the strains tested (except the *Strep. lactis* and *cremoris* strains) were selected for their ability to curdle milk in 24 hr. at 25° C. and, at the same time, to produce acetoin detectable by the Voges-Proskauer reaction. Other strains were included because of this latter property alone. Of thirty-four such strains, nine lost the ability to produce acetoin during the course of the investigation. These were strains nos. 53, 54, 100–105 and 112. They are, nevertheless, included in the table as 'similar strains' to the group 106. It is worth recording that these strains were not able to produce acetoin at the time of making

Table 1. *Cultural and biochemical reactions*

Strain	No. of similar strains (the described is included)	Litmus milk, 24 hr.	Haemolysis	Growth at 10° C.	Growth at 40° C.	Growth at 45° C.	Growth in presence of		Survival	Production of acid from																						Hydrolysis of arginine	Gelatin liquefaction	Production of CO_2	Production of acetoin from	
				Y.D.L. milk	D.L.B.	Y.D.L. milk	D.L.B.	D.L.B.	4% NaCl	6·5% NaCl	At pH 9·6	60° C. in D.L.B.	30 min. in Y.D.L. milk	Glycerol	Mannitol	Sorbitol	Xylose	Arabinose	Dextrose	Sucrose	Lactose	Maltose	Trehalose	Raffinose	Dextrin	Starch	Inulin	Salicin	Aesculin				Lactose	Lactose + citric acid
A 9	3	ARC	sl.g.	+	+	+	+	−	+	−	−	−	+	−	−	−	−	+	+	+	+	+	+	−	−	−	+	+	+	−	−	−	−	−	−
52	2	ARC	sl.g.	+	+	+	+	−	+	−	−	−	+	−	−	−	−	+	+	+	+	+	+	−	−	−	+	+	+	−	−	−	−	−	−
B 1	1	ARC	sl.g.	+	+	+	+	−	+	−	−	−	+	−	−	−	−	+	+	+	+	+	+	−	−	−	+	+	+	−	−	−	−	−	−
B 3	1	ARC	sl.g.	+	+	+	+	−	+	−	−	−	+	−	−	−	−	+	+	+	+	+	+	−	−	−	+	+	+	−	−	−	−	−	−
B 5	1	ARC	sl.g.	+	+	+	+	−	+	−	−	−	+	−	−	−	−	+	+	+	+	+	+	−	−	−	+	+	+	−	−	−	−	−	−
106	7	ARC	sl.g.	+	+	+	+	−	+	−	−	−	+	−	−	−	−	+	+	+	+	+	+	−	−	−	+	+	+	−	−	−	−	−	−
107	2	ARC	None	+	+	+	+	−	+	−	−	−	+	−	−	−	−	+	+	+	+	+	+	−	−	−	+	+	+	−	−	−	−	−	−
108	1	ARC	None	+	+	+	+	−	+	−	−	−	+	−	−	−	−	+	+	+	+	+	+	−	−	−	+	+	+	−	−	−	−	−	−
110	6	ARC	None	+	+	+	+	−	+	−	−	−	+	−	−	−	−	+	+	+	+	+	+	−	−	−	+	+	+	−	−	−	−	−	−
55	2	ARC	v.sl.g.	+	+	+	+	−	+	−	−	−	+	−	−	−	−	+	+	+	+	+	+	−	−	−	+	+	+	−	−	−	−	−	−
C 26/40	1	ARC	g.	+	+	+	+	−	+	−	−	−	+	−	−	−	−	+	+	+	+	+	+	−	−	−	+	+	+	−	−	−	−	−	−
C 27/6	2	ARC	g.	+	+	+	+	−	+	−	−	−	+	−	−	−	−	+	+	+	+	+	+	−	−	−	+	+	+	−	−	−	−	−	−
23 C 5	1	ARC	v.sl.g.	+	+	+	+	−	+	−	−	−	+	−	−	−	−	+	+	+	+	+	+	−	−	−	+	+	+	−	−	−	−	−	−
30 C 1	1	ARC	v.sl.g.	+	+	+	+	−	+	−	−	−	+	−	−	−	−	+	+	+	+	+	+	−	−	−	+	+	+	−	−	−	−	−	−
33 C 1	1	ARC	v.sl.g.	+	+	+	+	−	+	−	−	−	+	−	−	−	−	+	+	+	+	+	+	−	−	−	+	+	+	−	−	−	−	−	−
Strep. citrophilus 21	1	ARC	sl.g.	+	+	+	+	−	+	−	−	−	+	−	−	−	−	+	+	+	+	+	+	−	−	−	+	+	+	−	−	−	−	−	−
Strep. citrophilus 24	1	ARC	None	+	+	+	+	−	+	−	−	−	+	−	−	−	−	+	+	+	+	+	+	−	−	−	+	+	+	−	−	−	−	−	−
Strep. citrophilus RM1	1	ARC	None	+	+	+	+	−	+	−	−	−	+	−	−	−	−	+	+	+	+	+	+	−	−	−	+	+	+	−	−	−	−	−	−
Strep. lactis OJ	1	ARC	sl.g.	+	+	+	+	−	+	−	−	−	+	−	−	−	−	+	+	+	+	+	+	−	−	−	+	+	+	−	−	−	−	−	−
Strep. lactis Rice	1	ARC	sl.g.	+	+	+	+	−	+	−	−	−	+	−	−	−	−	+	+	+	+	+	+	−	−	−	+	+	+	−	−	−	−	−	−
Strep. lactis H 201	1	ARC	None	+	+	+	+	−	+	−	−	−	+	−	−	−	−	+	+	+	+	+	+	−	−	−	+	+	+	−	−	−	−	−	−
Strep. lactis M 3	1	ARC	sl.g.	+	+	+	+	−	+	−	−	−	+	−	−	−	−	+	+	+	+	+	+	−	−	−	+	+	+	−	−	−	−	−	−
Strep. cremoris H.P.	1	ARC	. . .	+	+	+	+	−	+	−	−	−	+	−	−	−	−	+	+	+	+	+	+	−	−	−	+	+	+	−	−	−	−	−	−
Strep. cremoris R.W.	1	ARC	. . .	+	+	+	+	−	+	−	−	−	+	−	−	−	−	+	+	+	+	+	+	−	−	−	+	+	+	−	−	−	−	−	−
Strep. cremoris 95/2	1	ARC	. . .	+	+	+	+	−	+	−	−	−	+	−	−	−	−	+	+	+	+	+	+	−	−	−	+	+	+	−	−	−	−	−	−

the other biochemical tests, but they showed no other differences from strains of the same origin which still produced acetoin.

Acetoin formation is closely connected with the utilization of citric acid. No trace of C_4-compounds or formation of visible amounts of CO_2 was detected in media containing only dextrose or lactose as carbon sources, but when both sugar and citrate were present strong gas production and formation of acetoin was observed. Further, strains A2, A9, B1, B3 and B5 were shown to break down the citric acid in milk with production of acetoin (Table 2), as with mixed culture starters containing *Strep. lactis* or *cremoris* and *Leuconostoc* species (34). This has also been observed with *Strep. citrophilus*, strain 21 (2). It was assumed that the strains which were not tested for citric acid breakdown behave similarly. Strains A2, A9, B1, B3 and B5 produce considerable amounts of volatile acids (other than CO_2) in milk (34).

The lactic acid formed in milk by the latter strains was shown to be dextrorotatory (34), but the type of lactic acid produced by other strains was not determined.

Table 2 *Citric acid content of* 48 *hr. old plain milk cultures. Original citric acid concentration*, 0·145%

Strain	pH	Citric acid (%)	Acetoin
A2	4·8	0·00	+
A9	4·8	0·00	+
B1	4·7	0·11	+
B3	4·6	0·09	+
B5	4·9	0·00	+

Serological results

In a preliminary series of precipitin ring tests extracts of all strains were found to precipitate with a group N serum, but a group D serum gave negative results. The following strains, all of different origin, were then selected for preparation of type and group sera: strains nos. 52, A9, 106, C26/40 and C27/6 fermented citric acid. Two strains, 'O.J.' and 'Rice', of *Strep. lactis* and strain 'H.P.', of *Strep. cremoris* were also included.

Serological types

When the agglutination titre, after three to six inoculations, was sufficiently high, type-specific sera were prepared by absorbing the sera with cross-reacting heterologous strains, which had been shown to belong to different types by reciprocal absorption and 5-tube agglutinin tests. Eight serological types were differentiated.

In direct (slide) agglutinin tests with suspensions of all strains included in this work the type sera were found to react in the following way (the types are named after the homologous organism):

Type A9	Agglutinates strains 52, 56, 57, A2, A9
Type 106	Agglutinates strains 53, 100–114
Type C26/40	Agglutinates homologous strain
Type C27/6	Agglutinates homologous strain
Type 23 C5	Agglutinates homologous strain
Type *Strep. lactis* O.J.	Agglutinates homologous strain
Type *Strep. lactis* Rice	Agglutinates homologous strain and strains B1, B3, B5
Type *Strep. cremoris* H.P.	Agglutinates homologous strain

Strains 54 and 55, the *Strep. citrophilus* strains, and 23 C5, 30 C1, 33 C1, did not react with any of the above sera.

As neither reciprocal nor one-sided absorption tests were made the above-mentioned positive agglutinin reactions can only give a certain probability that the reacting strains belong to the corresponding types.

In most cases strains of different origin belong to different types, but those of the same origin were not always serologically identical. For example, amongst strains 52–57, isolated from the same starter culture, at least three types are represented. It is worth mentioning that strains 100–105 and 107–114, whether producing acetoin or not, all reacted with type serum 106. The acetoin-producing strains B1–B5 reacted with *Strep. lactis* (Rice) type serum.

Group sera

Potent anti-sera reacting with all the above-mentioned types in precipitin ring tests were obtained after eleven to nineteen inoculations with strains nos. 52, A9, C26/40, C27/6, and with *Strep. lactis* strains 'O.J.' and 'Rice.'

The specificity of these sera was established by testing against extracts of representatives of each of the Lancefield groups A–N and with a strain of a staphylococcus, rich in the unspecific nucleo-protein factor common to all streptococci and staphylococci (Lancefield(16)). In these tests all sera reacted within 5 min. with group N extracts.

The serum against C27/6 also reacted with the staphylococcus extract and with all of the Lancefield groups except A, B and F. This serum was rendered specific by absorption with the staphylococcus strain. The absorbed serum was used for the reciprocal absorption tests and for the precipitin tests with extracts of the other strains in this work.

One of the sera obtained with *Strep. lactis* O.J. gave a slight cross-reaction after 30 min. with extracts of a group E streptococcus. The serum obtained from another rabbit was specific.

The sera prepared with strains 52, A9, C26/40 and *Strep. lactis* Rice, were specific.

The sera obtained were clearly group and this was confirmed by their precipitation with extracts of nine heterologous group N strains comprising at least four different types (p. 261 and reference(27)). The strains were *Strep. lactis* strains 'O.J.' and 'Rice', M1 and H201 and *Strep. cremoris* strains H.P., R.W., 135/5, and 95/2.

In spite of the fact that a group N serum has not yet been prepared with *Strep. cremoris*, it is assumed that this organism belongs to group N, as suggested by Shattock & Mattick(28). Attempts to prepare a *Strep. cremoris* serum in this investigation did not give clear-cut results. One serum was obtained which reacted with extracts of the *Strep. cremoris* strains just mentioned and with extracts of the acetoin-producing organisms, but this serum gave only very weak reactions with *Strep. lactis* extracts. When, after a prolonged course of inoculations, the reactions with *Strep. lactis* were considered strong enough, the serum also reacted strongly with group K and L extracts. It seems, however, reasonable to regard *Strep. cremoris* as a group N organism at present, but further work is needed to settle the point.

Reciprocal absorption tests

The serological identity with group N of the citric acid fermenting streptococci defined earlier, as well as of *Strep. lactis* Rice, was confirmed in reciprocal absorption tests with sera for these strains and a serum for *Strep. lactis* O.J. *Strep. lactis* O.J. belongs to group N, according to definition(28,3).

The results of these tests are given in Tables 3 and 4.

All the precipitin tests in these tables were made as ring tests and were confirmed by the Lancefield technique (17). The results leave no doubt that the relevant organisms belong to group N.

Reciprocal absorption tests, with ring tests only, were also made with the following pairs of sera: *Strep. lactis* Rice and strain A 9; *Strep. lactis* Rice and strain C 26/40; strain A 9 and strain C 27/6.

Table 3. *Absorption tests with group N serum* (Streptococcus lactis *O.J.*)

		Serum *Strep. lactis* O.J. absorbed with					
Extract of	Unabsorbed	O.J.	Rice	A 9	C 26/40	C 27/6	*Strep. durans* 98 D
Strain A 9	+	–	–	–	–	–	+
Strain 106	+	–	–	–	–	–	+
Strain C 26/40	+	–	–	.	–	–	+
Strain C 27/6	+	–	–	–	–	–	+
Strep. lactis O.J.	+	–	+	+	+	+	+
Strep. lactis Rice	+	–	–	–	–	–	+
Strep. lactis H 201	+	–	–	–	–	–	+
Strep. lactis M 3	+	–	–	.	.	.	+
Strep. cremoris H.P.	+	–	–	–	–	–	+
Strep. cremoris R.W.	+	–	–	.	.	.	+
Strep. cremoris J.	+	–	–	–	–	–	+
Strep. cremoris 95/2	+	–	–	.	.	.	+

Table 4. *Absorption tests with new sera.* (Streptococcus lactis *O.J. used for absorption*)

	Serum *Strep. lactis* Rice			Serum strain A 9			Serum Strain C 26/40			Serum strain C 27/6		
		Absorbed with strain			Absorbed with strain			Absorbed with strain			Absorbed with strain	
Extract of	Unabsorbed	O.J.	98 D	Unabsorbed	O.J.	98 D	Unabsorbed	O.J.	98 D	Unabsorbed	O.J.	98 D
Strain A 9	+	–	+	+	+	+	+	–	+	+	–	+
Strain 106	+	–	+	+	–	+	+	–	+	+	–	+
Strain C 26/40	+	–	+	+	.	.	+	+	+	+	–	+
Strain C 27/6	+	–	+	+	–	+	+	–	+	+	+	+
Strep. lactis O.J.	+	–	+	+	–	+	+	–	+	+	–	+
Strep. lactis Rice	+	+	+	+	–	+	+	–	+	+	+	+
Strep. lactis H 201	+	–	+	+	.	.	+	–	+	+	–	+
Strep. lactis M 3	+	–	+	+	.	.	+	–	.	+	.	+
Strep. cremoris H.P.	+	–	+	+	–	+	+	–	+	+	–	+
Strep. cremoris R.W.	+	–	+	+	.	.	+	.	.	+	–	+
Strep. cremoris J.	+	–	+	+	–	+	+	–	+	+	–	+
Strep. cremoris 95/2	+	–	+	+	.	.	+	.	.	+	.	+

One-sided absorption tests were made with the same technique on sera for strain A 9 with *Strep. lactis* Rice, for strain C 27/6 with *Strep. lactis* Rice, and for strain C 26/40 with strain A 9.

The results of these tests are not given in detail, but they confirmed that these organisms belong to the same group.

Precipitin tests with the citric acid fermenting streptococci

Potent and group-specific sera for strain A 9, C 26/40, C 27/6, as well as for *Strep. lactis* Rice and O.J., all shown to belong to group N, gave clear-cut precipitates with extracts of all strains included in this investigation. None of these extracts reacted with a serum for *Strep. bovis* Pearl 11, nor with a serum for *Strep. durans* 98 D, both group D strains (25,31).

DISCUSSION

Homogeneity of strains

The citric acid fermenting strains dealt with in this paper share most of the biochemical and cultural reactions, and form a fairly homogeneous group of organisms. There is, however, some variation. Some strains do not grow at 40° C., and the same strains also fail to grow in the presence of 4% NaCl. A few strains are able to multiply in 6·5% NaCl. Two strains survive 60° C. for 30 min. in D.L.B., and about half of them survive the same treatment in milk. This is not surprising as it is well known (21) that milk as a medium is more protective than broth, and, further, the inoculum in the Demeter test (milk) is bigger than in the standard D.L.B. test. Acid production from mannitol and sorbitol is variable, and sucrose is fermented only by a few strains, which also produce acid from raffinose. Arginine is hydrolysed by some of the strains.

None of these differences justifies division into biochemical subgroups.

Relation to older species

As mentioned earlier, many investigators have isolated and defined, more or less clearly, strains of citric acid fermenting streptococci other than *Leuconostoc*. One of these, *Strep. citrophilus* van Beynum & Pette (1), was compared directly with regard to its properties, with the other strains investigated in this paper. There is nothing in the serological or biochemical reactions to justify the view that *Strep. citrophilus* is distinct from the other strains investigated. In other cases, however, it is necessary to consider the data given in the relevant literature. According to the description given by Matuszewski *et al.* (22) *Strep. diacetilactis* grows at 10 and 40° C., but not at 45° C. It survives 60° C. for 30 min. in the Demeter test. Further, it produces D-rotatory lactic acid in milk, with CO_2 and C_4-compounds in considerable quantity. It does not ferment glycerol, mannitol, xylose, sucrose, raffinose or inulin. Acid production from arabinose is weak, but in dextrose, lactose, maltose, dextrin and salicin it is strong. It seems from these data that there is good reason to regard *Strep. diacetilactis* as identical with *Strep. citrophilus* and with the other strains investigated here.

The so-called i-bacteria of Knudsen & Sørensen (15) differ only slighly from *Strep. lactis* in respect of sugar fermentations, but produce CO_2 in milk (this property is not constant on subculturing). The description of the i-bacteria, though suggestive, is not sufficient to warrant a definite conclusion as to their identity with the citric acid fermenting strains.

Sørensen (33) has isolated and investigated many citric acid fermenting streptococci, which he, probably correctly, identified with *Strep. diacetilactis*. Sørensen states that these strains produce much acetoin but only a little diacetyl. His analyses, however, referred only to fully ripened cultures, in which the diacetyl may have been reduced. Some strains investigated in this paper have been shown to produce up to 20 mg. diacetyl per litre culture in milk at the time of the disappearance of the citrate. The diacetyl is then rapidly reduced and the final level in a 24 hr. culture is about 0·5–2 mg. diacetyl per litre (34).

Karnad (14) and Joshi & Ram Ayyar (13) report on two species, *Strep. diacetyl aromaticus* and *Strep. lactis aromaticus*. As far as can be judged from the very short descriptions given, there is no reason to regard these species as different. It is probable that these organisms are identical with the strains investigated in this paper.

It must therefore be concluded that the streptococci described in the earlier literature, which rapidly produce D-lactic acid in milk with simultaneous breakdown of citrate and

production of CO_2, volatile acids and C_4-compounds, are so similar as to constitute a taxonomic unit which includes the strains dealt with in this paper.

Relationship to the enterococci

Members of the unit, as defined above, have been regarded as related to the enterococci (6,35), but the serological results reported here show clearly that there is in fact no such relationship. This is confirmed by the results of the cultural and biochemical tests. It is true that some strains grow in the presence of 6·5 % NaCl and survive heating at 60° C. for 30 min., but these are the only respects in which they may be said to resemble the enterococci.

Relationship to lactis-cremoris group

The relationship with the *Strep. lactis-cremoris* group, on the other hand, is serologically clearly established. With regard to cultural and biochemical reactions the main difference is the utilization of citric acid in the production of gas and other products. (The production of these compounds in milk does not, however, warrant the term heterofermentative used in the accepted sense, as it is not a pure carbohydrate fermentation.) In other respects most of the strains are most similar to *Strep. lactis*; only a few of them bear some resemblance to *Strep. cremoris* (e.g. strain 23 C5, no growth at 40° C., no growth in 4 % NaCl, no arginine hydrolysis), but do not possess all the characteristics of this organism (e.g. the strains mentioned produced acid from maltose and dextrin (12,18,30)). The suggestion of Bergey (3) that *Strep. diacetilactis* Matuszewski *et al.* (22) is the same as *Strep. cremoris* O.J. cannot be accepted.

It may, however, be considered whether the relevant unit should be regarded as different from *Strep. lactis*. The differentiating character, the power to ferment citrate, is sharp enough for the two units to appear distinctly different, but it has been frequently reported in the literature (6,22,24,35) that citrate fermenting strains may lose this character on subculturing. This question will have to be investigated further as it is very important both from the theoretical and practical points of view. For the moment the following facts may be taken into account. In the present investigation some of the strains studied, because of their ability to produce acetoin, lost this ability in the course of the investigation. However, it cannot be denied that some confusion may have been caused by the difficulty of isolating pure cultures of these diplococci or chain-forming organisms, possibly giving erroneous results, especially as it has been possible to maintain cultures which retain the power to ferment citrate for several years (compare (23), and the fact that the strains in this paper were isolated in 1948 and strain P21, the *Strep. citrophilus* type strain, was isolated in 1936 and is still a vigorous gas-former). Moreover, it is well known from dairy practice, where these strains are sometimes used in starter cultures, that they may lose the ability to form gas and acetoin. This is, however, probably a different problem, as this alteration in the cases investigated (34) has been found to accompany a general decrease in viability, not uncommon in starter cultures. It therefore seems most probable that the relevant citrate fermenting strains, at least on careful subculturing in suitable media, retain their characteristic properties and thus are distinctly different from *Strep. lactis*.

Whether the citrate fermenting unit is worthy of species rank or not is largely dependent upon what weight should be attached to the power to ferment citrate. There is no generally accepted rule defining what the differences should be between taxonomic units to justify a distinction as species. As in this particular case the differentiating character is very

sharp, and in respect of the fermentation products of the citrate fermentation rather important, it seems correct to regard *Strep. lactis* O.J. and the citrate fermenting unit as two distinct species.

If, on the other hand, in the future one single biochemical character should be regarded as insufficient to differentiate between two species—as is now the tendency in modern bacteriology—this question will have to be raised again. Moreover, it will have to be discussed from a somewhat broader point of view, namely in connexion with a general discussion of the taxonomic position of all the units, now regarded by Bergey(3) as varieties of *Strep. lactis* O.J., i.e. *Strep. lactis* var. *maltigenes*, *Strep. lactis* var. *anoxyphilus*, *Strep. amylolactis*, etc., as well as *Strep. cremoris* O.J. It might well be that after a thorough investigation of all these units it will be found convenient to group at least some of them, including perhaps the citrate fermenting unit, as subspecies (or 'varieties') of *Strep. lactis*.

Nomenclature

From the point of view of priority the following three specific epithets for the relevant units are possible: *diacetilactis* Matuszewski *et al.*(22), *citrophilus* van Beynum & Pette(1) and *lactis aromaticus* Joshi & Ram Ayyar(13), all described in 1936. The last name may be discarded at once as it is not in accordance with the recommendations of the 1935 rules of botanical nomenclature (Bergey(3)). The epithet *citrophilus*, though descriptive, has the disadvantage of not being specific, as other streptococci are known which possess the power to ferment citrate. The epithet *diacetilactis* is thus left and the citrate fermenting unit described in this paper should therefore retain the name *Strep. diacetilactis* given by Matuszewski *et al.*

SUMMARY

1. Thirty-five strains of acetoin producing lactic streptococci isolated from raw milk, from starter cultures and from dairy products were investigated biochemically and serologically.

2. The strains are shown to form a homogeneous unit, very similar to *Strep. lactis* O.J., which species they resemble in cultural behaviour, in growth-temperature range, in tolerance of sodium chloride and heat and in carbohydrate and other fermentations. They differ from *Strep. lactis* essentially in their ability to utilize citrate in producing CO_2 volatile acids and C_4-compounds (diacetyl, etc.). With few exceptions they bear no resemblance to the enterococci.

3. Serologically the strains are shown to belong to group N and thus the relationship to *Strep. lactis* is clearly established.

4. The strains investigated are shown to be identical with *Strep. citrophilus* van Beynum & Pette and almost with certainty, though no direct comparison has yet been possible, with *Strep. diacetilactis* Matuszewski *et al.** Probably they are identical, too, with *Strep. diacetyl aromaticus* Karnad and *Strep. lactis aromaticus* Joshi & Ram Ayyar.

5. The taxonomic position of the relevant unit is discussed, and it is suggested that it be given species rank under the name *Strep. diacetilactis*.

* *Note added* 16 *April* 1951—A freshly isolated culture of *Strep. diacetilactis* recently received, through the kind offices of Prof. Eug. Pijanowski, Szkola Glowna Gospodarstwa Wiejskiego, Zaklad Technologii Zywnosci, Warsaw, from Dr J. Supinska-Jakubowska, 95, Narutowicza, Lodz, Poland, has now been shown by Dr C. A. E. Briggs, of the National Institute for Research in Dairying to belong to serological group N.

To Dr A. T. R. Mattick I wish to render my most sincere thanks for the excellent working conditions afforded me, for his great interest in my work and for valuable advice. I also thank him for kindly reviewing this paper. I am greatly obliged to Dr C. L. Hannay, who spared no effort to give me a good start in my work, and to the staff of the N.I.R.D. for informative discussions and for very good technical assistance.

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452. BACTERIOLOGICAL TESTS FOR MILK SAMPLED DURING DISTRIBUTION TO THE CONSUMER

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(With 1 Figure)

In England and Wales different tests are now in use officially for the examination of raw and pasteurized milk sampled at the time of distribution to the consumer. Designated raw milk is examined by the methylene-blue test at 37° C. within 2 hr. of sampling, and samples must not reduce the dye in 4½ hr. between 1 May and 30 October, and in 5½ hr. between 1 November and 30 April (1). In addition to a phosphatase test, a methylene-blue test at 37° C. is also used to control pasteurized milk, but the samples are stored at shade temperature until between 9 and 10 a.m. on the day after distribution, and must then not reduce the dye within ½ hr.; if the maximum temperature during the storage period exceeds 65° F., test results are void (2).

From the consumer's point of view there is little justification for the use of different tests and standards for raw and pasteurized milks. The consumer, being assured of a safe milk supply, requires the milk he receives to remain palatable and uncurdled until it is used, generally 24 hr. after delivery. It would seem, therefore, that the interest of the consumer would be served if one test, showing that the milk he receives is of satisfactory keeping quality, were to be applied to samples taken during distribution.

Extensive investigations under the direction of a committee appointed by the Ministry of Agriculture and Fisheries (3,4,5) have indicated that dye tests are not sufficiently reliable as measures of the keeping quality of raw milk samples. As an alternative, a clot-on-boiling (C.O.B.) test applied after storage of samples under standard conditions is suggested as an objective measure of keeping quality. A method for the grading of farm milk supplies, based on this test, has been outlined (4), and results obtained in an extensive field trial with this and other standard methods have been given (5).

Since, for the official control of all milk sampled during distribution, it seemed desirable to use a single test to indicate the actual keeping quality of milk in the hands of the consumer, the Ministry of Food asked Dr G. S. Wilson, of the Public Health Laboratory Service, and Dr A. T. R. Mattick and Mr A. Rowlands, of the National Institute for Research in Dairying, to carry out an investigation and report on the results.

After preliminary discussions, it was decided to make a survey of both raw and pasteurized milk, sampled during distribution, in as many areas as possible throughout England and Wales. The results obtained in the survey form the basis of this report.

EXPERIMENTAL

Six commercial dairy laboratories, twenty-four P.H.L.S. (Public Health Laboratory Service) laboratories, and fifteen N.A.A.S (National Agricultural Advisory Service) and N.M.T.S. (National Milk Testing Service) laboratories collaborated in the trials which were

carried out in many areas throughout England and Wales. All samples were taken and delivered to the testing laboratories on the day of distribution of the milk to consumers. Four types of distributors were sampled as follows:

A. Producer retailers distributing their own designated *raw* milk.
B. Dairies distributing designated *raw* milk received from farms.
C. Dairies distributing their own *heat-treated* milk.
D. Dairies distributing *heat-treated* milk processed at other dairies.

The P.H.L.S. laboratories tested samples, taken officially, from each of the four types of distributors: a random selection of distributors was, however, not possible. The commercial dairies were all type C distributors and tested samples of their own heat-treated milk; one, type B also, tested samples of designated raw milk.

Each of the N.A.A.S. and N.M.T.S. laboratories made a random selection of type C and D distributors to be sampled. Having chosen not less than eight and not more than twenty distributors of one type which were convenient for sampling, a number was assigned to each, and four of the numbered dairies were then selected for sampling in the order they appeared on a card of random numbers (1–20) sent to each centre taking part. This process was carried out once for the selection of C and once for the selection of D distributors, using different random order cards for each.

Treatment and testing of samples

On receipt of the samples in the laboratory the necessary number of 10 ml. quantities—four for the A and B, and three for the C and D dairies—were transferred aseptically to sterile $6 \times \frac{3}{8}$ in. test-tubes closed with sterile rubber bungs. One tube was then used for tests L, M, N, O or P.

Test L: methylene-blue ($4\frac{1}{2}/5\frac{1}{2}$ *hr.*) *test.* This test is used officially for the examination of designated raw milk in England and Wales (1). For this test, one tube of milk was stored in the ice-chest until 9–9.30 a.m. on the day after sampling and then, after the addition of the methylene blue, incubated at 37–38° C. (1). Samples not decolorizing the dye in $4\frac{1}{2}$ hr. between 1 May and 31 October, and in $5\frac{1}{2}$ hr. between 1 November and 30 April were deemed to have passed the test.

Test M: methylene-blue ($\frac{1}{2}$ *hr.*) *test* (*shade storage*). This test is used officially for testing pasteurized milk in England and Wales (2). For this test, one tube of milk was stored at shade atmospheric temperature until 9–9.30 a.m. on the day after sampling and, after the addition of the methylene blue, incubated at 37–38° C. (2). Samples not decolorizing the dye in $\frac{1}{2}$ hr. were deemed to have passed the test.

Test N: methylene-blue ($\frac{1}{2}$ *hr.*) *test* (20° C. *storage*). For this test, one tube of milk was stored at shade atmospheric temperature until 3.30–4 p.m. on the day of sampling, and thereafter at 20 ± 1° C. until 9–9.30 a.m. the next morning. The dye was then added and the tube incubated at 37–38° C. Samples not decolorizing the dye in $\frac{1}{2}$ hr. were deemed to have passed the test.

Test O: clot-on-boiling test (*C.O.B. shade storage*). For this test one tube of milk was stored exactly as described for test M until 9–9.30 a.m. on the day after sampling. After shaking the tube, about 2 ml. of milk was transferred to a clean test-tube which was placed in boiling water for 5 min. Samples showing no sign of precipitation were deemed to have passed the test.

Test P: clot-on-boiling test (*C.O.B.* 20° C. *storage*). For this test one tube of milk was

stored at shade atmospheric temperature until 3.30–4 p.m. on the day of sampling, and thereafter at 20 ± 1° C. until 9–9.30 a.m. the following morning. The C.O.B. test was then done as for test O. Samples showing no sign of precipitation were deemed to have passed the test.

The tests actually done on the samples of designated raw milk from A and B dairies, and on the heat-treated milk from C and D dairies in summer and winter are shown in Table 1. The same tests were carried out on samples from all dairies, except that only samples of designated raw milk from the A and B dairies were examined by the methylene-blue ($4\frac{1}{2}/5\frac{1}{2}$ hr) test (L).

Table 1. *Tests applied in summer and winter to samples from dairies A and B, and dairies C and D*

	Methylene-blue			Clot-on-boiling (C.O.B.)	
	$4\frac{1}{2}/5\frac{1}{2}$ hr. (0–4° C. storage)	$\frac{1}{2}$ hr. (shade storage)	$\frac{1}{2}$ hr. (20° C. storage)	Shade storage	20° C. storage
	Designated raw milk, dairies A and B				
May–November 1949	L	M	No test	O	P
December 1949–April 1950	L	M	N	No test	P
	Heat-treated milk, dairies C and D				
May–November 1949	No test	M	No test	O	P
December 1949–April 1950	No test	M	N	No test	P

RESULTS

After preliminary work in some laboratories, the trials were begun in May 1949 and ended in April 1950. It was decided to exclude from the analysis of the results those samples which were not examined by all tests and those tested when the temperature of the 20° C. water-bath varied by more than ± 1° C. Of the 20,159 samples examined by all the appropriate tests, 2·8 and 0·5 % had to be discarded because the records showed the 20° C. water-bath temperatures to have been over 21 or under 19° C. respectively. The total number of samples analysed is shown in Table 2.

Table 2. *The total number of samples used in the analysis of the results*

Testing laboratory	Designated raw milk		Heat-treated milk	
	A dairies	B dairies	C dairies	D dairies
P.H.L.S.	1,214	1,245	10,367	2,378
Commercial dairies	0	125	2,773	0
N.A.A.S. and N.M.T.S.	0	0	727	670
Total	1,214	1,370	13,867	3,048

The results for each of the four types of distributor—A, B, C and D—have been analysed separately on the basis of the incidence of failures with each test. Information is also given on the relative performance of the six commercial dairies (C distributors) testing their own supplies, and a number of C and D distributors selected at random and tested by N.A.A.S. and N.M.T.S. laboratories.

The incidence of failures each month according to the maximum shade atmospheric temperature

Officially, results of the methylene-blue ($\frac{1}{2}$ hr.) test applied to samples of pasteurized milk are void if the maximum shade atmospheric temperature during the 24 hr. period

beginning at 9 a.m. on the day of sampling exceeds 65° F. (2). It was of interest, therefore, to determine the proportion of samples failing when the maximum shade atmospheric temperature was above or below 65° F.

During the trials, each laboratory recorded the maximum temperature in the cabinet used for shade storage of samples during the 24 hr. period beginning at 9 a.m. on the day of sampling. The results for each month have been classified according to the maximum shade temperature into the following four groups:

Group 1. Maximum shade temperature up to and including 65° F.
Group 2. Maximum shade temperature over 65° F. up to and including 70° F.
Group 3. Maximum shade temperature over 70° F. up to and including 75° F.
Group 4. Maximum shade temperature over 75° F.

The results for each type of distributor are given separately in Table 3. It may be seen that, with each test, the incidence of failing samples increased with increasing shade temperature. This is immediately apparent from a study of the totals given for each type of distributor. Thus for samples from C type distributors tested during May to November the percentage failures with the official methylene-blue (½ hr.) test (M) was 6·3% at temperatures up to 65° F., and 13·4, 20 and 40% at temperatures in the range 65–70, 70–75 and over 75° F. respectively.

The incidence of failures for samples tested at temperatures up to 65° F. is, of course, weighted by the inclusion of all results for tests below this temperature. Table 3 is therefore modified by re-grouping the temperatures as follows:

Group I. Maximum shade temperature up to and including 65° F.
Group II. Maximum shade temperature up to and including 70° F.
Group III. Maximum shade temperature up to and including 75° F.

The high incidence of failures with all tests when the maximum temperature exceeded 75° F. showed that further analysis in this range was unprofitable. From these new figures, Table 4, a more balanced impression may be gained of the monthly incidence of failures when a specified maximum temperature is not exceeded. It may now be seen that for C group distributors sampled in the period May to November, the percentage failures by the methylene-blue (½ hr.) test (M) was 8·3 and 9·5% at maximum temperatures of 70° F. or less and 75° F. or less. At maximum temperatures of 65° F. or less the figure, already quoted, is 6·3%.

The percentage failures for each test and for each type of distributor is lower during the winter period, December to April, than during the summer period, May to November. For the methylene-blue (½ hr.) test (M) the fewer failures are no doubt due, in part, to the lower prevailing shade temperatures during the 24 hr. pre-test storage period. This argument cannot be applied to the results of the methylene-blue (4½/5½ hr.) test (L), since the samples were stored in the ice-chest, and only partly to the results of the C.O.B. test (P), since these samples were stored at atmospheric shade temperature until 4 p.m. on the day of sampling and thereafter at 20° C. Clearly all distributors experienced less difficulty in complying with the standards of each test in winter than in summer. From this it may be inferred that milk at the time of distribution is, in fact, of a higher bacteriological quality in winter than in summer. Moreover, when atmospheric temperatures rise above 65° F., the difficulty experienced in complying with the standards imposed by each test is proportionately accentuated.

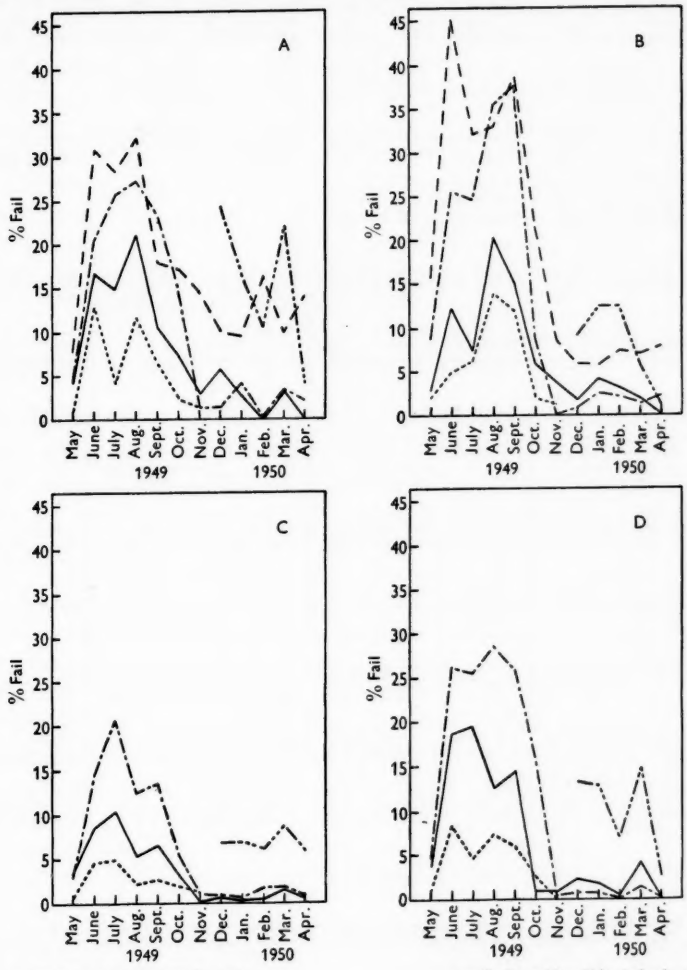
Table 3. *The percentage of failing samples with each test (per month)**

	Maximum atmospheric shade temperature—recordings made at 9 a.m. and 4 p.m. on day of sampling																					
	Over 75° F.						Over 70° F. up to and including 75° F.						Over 65° F. up to and including 70° F.						Up to and including 65° F.					
	L	M	N	O	P	Total samples	L	M	N	O	P	Total samples	L	M	N	O	P	Total samples	L	M	N	O	P	Total samples
Samples A. Designated raw milk from producer-retailers																								
1949 May	—	—	—	—	—	0	(0)	(1)	—	(0)	(1)	7	6·1	9·1	—	3·0	9·1	33	8·6	2·5	—	0·0	2·5	81
June	34·8	69·6	—	30·4	39·1	23	34·0	36·0	—	22·0	24·0	50	52·0	32·0	—	24·0	32·0	25	20·8	15·1	—	7·5	9·4	53
July	57·1	47·6	—	21·4	26·2	42	47·7	36·4	—	34·1	45·5	44	37·1	28·6	—	5·7	22·9	35	20·5	23·1	—	2·6	7·7	39
August	33·3	55·6	—	18·5	33·3	27	38·2	32·4	—	23·5	29·4	34	35·7	32·1	—	19·6	32·1	56	29·2	23·6	—	5·6	12·5	72
September	(1)	(0)	—	(0)	(0)	1	35·7	0·0	—	14·3	14·3	14	13·7	29·4	—	9·8	11·8	51	22·7	15·9	—	2·3	9·1	44
October	—	—	—	—	—	0	(1)	(1)	—	(0)	(0)	3	(3)	(2)	—	(0)	(1)	4	10·8	10·8	—	2·7	5·4	37
November	—	—	—	—	—	0	—	—	—	—	—	0	(0)	(0)	—	(0)	(0)	1	14·7	1·5	—	1·5	2·9	68
December	—	—	—	—	—	0	—	—	—	—	—	0	(0)	(0)	(0)	—	(0)	1	10·1	1·4	24·6	—	5·8	69
1950 January	—	—	—	—	—	0	—	—	—	—	—	0	(0)	(0)	(0)	—	(0)	1	9·7	4·2	16·7	—	2·8	72
February	—	—	—	—	—	0	—	—	—	—	—	0	—	—	—	—	—	0	16·3	0·0	10·5	—	0·0	86
March	—	—	—	—	—	0	—	—	—	—	—	0	—	—	—	—	—	0	9·9	3·3	22·0	—	3·3	91
April	—	—	—	—	—	0	—	—	—	—	—	0	—	—	—	—	—	0	14·0	2·0	4·0	—	0·0	50
May to November	45·2	54·8	—	22·6	31·2	93	37·5	30·9	—	23·7	29·6	152	28·3	27·3	—	12·2	21·5	205	18·0	12·2	—	3·0	6·9	394
December to April	—	—	—	—	—	0	—	—	—	—	—	0	(0)	(0)	(0)	—	(0)	2	12·0	2·2	16·3	—	2·4	368
Samples B. Designated raw milk from distributors																								
1949 May	—	—	—	—	—	0	(0)	(1)	—	(1)	(1)	7	14·8	7·4	—	7·4	3·7	27	16·0	9·3	—	0·0	2·7	75
June	62·5	81·3	—	12·5	18·8	16	56·0	24·0	—	20·0	24·0	25	43·2	25·0	—	4·5	15·9	44	47·4	26·3	—	5·3	7·9	38
July	55·3	57·8	—	34·2	42·1	38	38·5	26·9	—	15·4	15·4	26	36·1	27·8	—	5·6	11·1	36	28·9	22·2	—	6·7	4·4	45
August	55·0	65·0	—	35·0	25·0	20	41·2	23·5	—	23·5	23·5	17	36·1	36·1	—	16·7	16·7	36	30·2	34·9	—	11·6	23·3	43
September	(3)	(2)	—	(0)	(0)	3	(3)	(3)	—	(2)	(2)	6	37·0	33·3	—	22·2	18·5	27	39·2	39·2	—	8·1	13·5	74
October	—	—	—	—	—	0	(0)	(0)	—	(0)	(0)	2	34·6	19·2	—	3·8	7·7	26	17·1	5·3	—	1·3	5·3	76
November	—	—	—	—	—	0	—	—	—	—	—	0	(0)	(0)	—	(0)	(0)	1	8·8	0·0	—	1·3	3·8	80
December	—	—	—	—	—	0	—	—	—	—	—	0	—	—	—	—	—	0	5·9	0·8	9·2	—	1·7	119
1950 January	—	—	—	—	—	0	—	—	—	—	—	0	0·0	0·0	0·0	—	16·7	18	6·8	2·9	14·6	—	1·9	103
February	—	—	—	—	—	0	—	—	—	—	—	0	—	—	—	—	—	0	7·3	2·1	12·5	—	3·1	96
March	—	—	—	—	—	0	—	—	—	—	—	0	—	—	—	—	—	0	7·1	1·3	5·8	—	1·9	156
April	—	—	—	—	—	0	—	—	—	—	—	0	—	—	—	—	—	0	7·8	2·2	1·1	—	0·0	90
May to November	58·4	64·9	—	28·6	31·2	77	41·0	25·3	—	19·3	20·5	83	34·5	25·4	—	9·6	12·7	197	24·4	17·4	—	4·2	7·9	431
December to April	—	—	—	—	—	0	—	—	—	—	—	0	0·0	0·0	0·0	—	16·7	18	6·9	1·8	8·5	—	1·8	564
Samples C. Heat-treated milk distributed by processing dairy																								
1949 May	—	0·0	—	0·0	0·0	17	—	15·2	—	12·1	0·0	33	—	6·2	—	1·9	3·5	257	—	1·9	—	0·2	3·4	855
June	—	34·9	—	13·3	12·4	241	—	12·7	—	4·2	6·8	118	—	18·9	—	5·0	9·6	280	—	11·0	—	4·3	7·8	346
July	—	61·4	—	36·2	31·1	334	—	18·8	—	11·7	15·6	154	—	25·0	—	6·5	14·4	216	—	17·9	—	3·9	8·1	358
August	—	18·9	—	5·0	6·3	159	—	23·6	—	12·4	16·1	161	—	13·0	—	2·8	6·6	316	—	12·3	—	1·5	4·2	333
September	—	25·0	—	8·0	5·4	112	—	24·6	—	9·9	10·6	142	—	13·4	—	3·3	5·9	305	—	13·6	—	2·4	6·9	536
October	—	(0)	—	(0)	(0)	4	—	19·5	—	8·5	6·1	82	—	10·9	—	2·9	8·0	175	—	4·2	—	1·7	2·2	787
November	—	—	—	—	—	0	—	—	—	—	—	0	—	0·0	—	0·0	0·0	121	—	1·1	—	1·2	0·0	1192
December	—	—	—	—	—	0	—	—	—	—	—	0	—	0·0	0·0	—	0·0	15	—	1·0	6·9	—	0·6	1052
1950 January	—	—	—	—	—	0	—	—	—	—	—	0	—	0·0	0·0	—	0·0	23	—	0·6	6·9	—	0·2	1306
February	—	—	—	—	—	0	—	—	—	—	—	0	—	(0)	(0)	—	(0)	4	—	1·7	6·1	—	0·5	1253
March	—	—	—	—	—	0	—	—	—	—	—	0	—	0·0	0·0	—	0·0	11	—	1·8	8·8	—	1·5	1424
April	—	—	—	—	—	0	—	(0)	(0)	—	(0)	1	—	0·0	0·0	—	0·0	11	—	0·8	5·9	—	0·6	1033
May to November	—	40·0	—	19·6	17·3	867	—	20·0	—	9·9	11·3	690	—	13·4	—	3·4	7·2	1670	—	6·3	—	1·7	3·5	4407
December to April	—	—	—	—	—	0	—	(0)	(0)	—	(0)	1	—	0·0	0·0	—	0·0	64	—	1·2	7·0	—	0·7	6068
Samples D. Heat-treated milk distributed by other than processing dairy																								
1949 May	—	(0)	—	(0)	(0)	2	—	(0)	—	(1)	(1)	6	—	6·5	—	0·0	2·2	46	—	4·1	—	1·0	4·1	194
June	—	75·0	—	43·2	31·8	44	—	33·3	—	11·1	19·4	36	—	25·5	—	9·1	14·5	55	—	26·9	—	7·7	23·1	52
July	—	78·8	—	47·0	51·5	66	—	39·4	—	24·2	30·3	33	—	26·7	—	6·7	20·0	45	—	25·0	—	3·6	19·0	84
August	—	37·5	—	6·3	18·8	16	—	53·7	—	19·5	39·0	41	—	25·5	—	8·5	8·5	47	—	31·9	—	6·4	17·0	47
September	—	21·4	—	0·0	14·3	14	—	38·2	—	23·5	32·4	34	—	31·8	—	9·4	18·8	85	—	19·8	—	2·5	9·9	81
October	—	—	—	—	—	0	—	(5)	—	(0)	(0)	9	—	22·9	—	2·9	11·4	35	—	14·6	—	2·7	8·6	185
November	—	—	—	—	—	0	—	—	—	—	—	0	—	—	—	—	—	0	—	0·4	—	0·0	0·8	254
December	—	—	—	—	—	0	—	—	—	—	—	0	—	(1)	(4)	—	(1)	9	—	0·3	12·4	—	2·1	291
1950 January	—	—	—	—	—	0	—	—	—	—	—	0	—	0·0	20·0	—	0·0	20	—	0·7	12·4	—	2·0	306
February	—	—	—	—	—	0	—	—	—	—	—	0	—	—	—	—	—	0	—	0·0	7·0	—	0·4	285
March	—	—	—	—	—	0	—	—	—	—	—	0	—	—	—	—	—	0	—	1·4	14·7	—	4·1	442
April	—	—	—	—	—	0	—	—	—	—	—	0	—	(0)	(0)	—	(0)	3	—	0·0	2·8	—	0·0	179
May to November	—	66·2	—	35·9	37·3	142	—	40·9	—	18·2	28·3	159	—	24·3	—	6·7	13·4	313	—	11·4	—	2·1	7·8	897
December to April	—	—	—	—	—	0	—	—	—	—	—	0	—	2·9	23·5	—	2·9	34	—	0·6	10·9	—	2·1	1503

Fig. 1. [illegible] the results [illegible] failures [illegible] temperature

Fig. 1 [illegible] failures exceed [illegible] These d[illegible]

Fig. 1 is a frequency polygon, drawn from the figures in Table 4, showing the percentage failures, in each month, for samples tested when the maximum shade temperature did not exceed 70° F. A separate diagram is given for the results from A, B, C and D distributors. These diagrams illustrate clearly the relative differences between each type of distributor;



———, methylene-blue ($4\frac{1}{2}/5\frac{1}{2}$ hr.) (L); — · —, methylene blue ($\frac{1}{2}$ hr.) shade storage (M);
— · · —, methylene-blue ($\frac{1}{2}$ hr.) 20° C. storage (N); · · · · · , clot-on-boiling shade storage (O);
————, clot-on-boiling 20° C. storage (P).

A, producer-retailer samples; B, distributor samples;
C, samples distributed by processing dairy; D, samples distributed by other than processing dairy.

Fig. 1. Percentage failures per month of designated raw milk (A and B) and of pasteurized milk (C and D) samples—maximum atmospheric shade temperature recorded 70° F. or less.

the relative incidence of failures with each test; the rise and fall in the proportion of failures each month and indirectly the main temperature effects following the change of temperature with the time of year.

Table 4. *The percentage of failing samples with each test (per month)**

	Maximum atmospheric shade temperature between 9 a.m. and 4 p.m. on day of sampling																	
	Up to and including 75° F.						Up to and including 70° F.						Up to and including 65° F.					
	L	M	N	O	P	Total samples	L	M	N	O	P	Total samples	L	M	N	O	P	Total samples
Samples A. Designated raw milk from producer-retailers																		
1949 May	7·4	5·0	—	0·8	5·0	121	7·9	4·4	—	0·9	4·4	114	8·6	2·5	—	0·0	2·5	81
June	32·0	26·6	—	16·4	19·5	128	30·8	20·5	—	12·8	16·7	78	20·8	15·1	—	7·5	9·4	53
July	35·6	29·7	—	15·3	26·3	118	28·4	25·7	—	4·1	14·9	74	20·5	23·1	—	2·6	7·7	39
August	33·3	28·4	—	14·2	22·8	162	32·0	27·3	—	11·7	21·1	128	29·2	23·6	—	5·6	12·5	72
September	20·2	20·2	—	7·3	11·0	109	17·9	23·2	—	6·3	10·5	95	22·7	15·9	—	2·3	9·1	44
October	18·2	15·9	—	2·3	6·8	44	17·1	14·6	—	2·4	7·3	41	10·8	10·8	—	2·7	5·4	37
November	14·5	1·4	—	1·4	2·9	69	14·5	1·4	—	1·4	2·9	69	14·7	1·5	—	1·5	2·9	68
December	10·0	1·4	24·3	—	5·7	70	10·0	1·4	24·3	—	5·7	70	10·1	1·4	24·6	—	5·8	69
1950 January	9·6	4·1	16·4	—	2·7	73	9·6	4·1	16·4	—	2·7	73	9·7	4·2	16·7	—	2·8	72
February	16·3	0·0	10·5	—	0·0	86	16·3	0·0	10·5	—	0·0	86	16·3	0·0	10·5	—	0·0	86
March	9·9	3·3	22·0	—	3·3	91	9·9	3·3	22·0	—	3·3	91	9·9	3·3	22·0	—	3·3	91
April	14·0	2·0	4·0	—	0·0	50	14·0	2·0	4·0	—	0·0	50	14·0	2·0	4·0	—	0·0	50
May to November	24·8	20·1	—	9·7	15·4	751	21·5	17·4	—	6·2	11·9	599	18·0	12·2	—	3·0	6·9	394
December to April	11·9	2·2	16·2	—	2·4	370	11·9	2·2	16·2	—	2·4	370	12·0	2·2	16·3	—	2·4	368
Samples B. Designated raw milk from distributors																		
1949 May	14·7	9·2	—	2·8	3·7	109	15·7	8·8	—	2·0	2·9	102	16·0	9·3	—	0·0	2·7	75
June	47·7	25·2	—	8·4	15·0	107	45·1	25·6	—	4·9	12·2	82	47·4	26·3	—	5·3	7·9	38
July	33·6	25·2	—	8·4	9·3	107	32·1	24·7	—	6·2	7·4	81	28·9	22·2	—	6·7	4·4	45
August	34·4	33·3	—	15·6	20·8	96	32·9	35·4	—	13·9	20·3	79	30·2	34·9	—	11·6	23·3	43
September	39·3	38·3	—	13·1	15·9	107	38·6	37·6	—	11·9	14·9	101	39·2	39·2	—	8·1	13·5	74
October	21·2	8·7	—	1·9	5·8	104	21·6	8·8	—	2·0	5·9	102	17·1	5·3	—	1·3	5·3	76
November	8·6	0·0	—	1·2	3·7	81	8·6	0·0	—	1·2	3·7	81	8·8	0·0	—	1·3	3·8	80
December	5·9	0·8	9·2	—	1·7	119	5·9	0·8	9·2	—	1·7	119	5·9	0·8	9·2	—	1·7	119
1950 January	5·8	2·5	12·4	—	4·1	121	5·8	2·5	12·4	—	4·1	121	6·8	2·9	14·6	—	1·9	103
February	7·3	2·1	12·5	—	3·1	96	7·3	2·1	12·5	—	3·1	96	7·3	2·1	12·5	—	3·1	96
March	7·1	1·3	5·8	—	1·9	156	7·1	1·3	5·8	—	1·9	156	7·1	1·3	5·8	—	1·9	156
April	7·8	2·2	1·1	—	0·0	90	7·8	2·2	1·1	—	0·0	90	7·8	2·2	1·1	—	0·0	90
May to November	29·1	20·5	—	7·5	10·7	711	27·5	19·9	—	5·9	9·4	628	24·4	17·4	—	4·2	7·9	431
December to April	6·7	1·7	8·2	—	2·2	582	6·7	1·7	8·2	—	2·2	582	6·9	1·8	8·5	—	1·8	564
Samples C. Heat-treated milk distributed by processing dairy																		
1949 May	—	3·2	—	1·0	3·3	1145	—	2·9	—	0·6	3·4	1112	—	1·9	—	0·2	3·4	855
June	—	14·2	—	4·6	8·3	744	—	14·5	—	4·6	8·6	626	—	11·0	—	4·3	7·8	346
July	—	20·2	—	6·3	11·5	728	—	20·6	—	4·9	10·5	574	—	17·9	—	3·9	8·1	358
August	—	14·8	—	4·2	7·5	810	—	12·6	—	2·2	5·4	649	—	12·3	—	1·5	4·2	333
September	—	15·2	—	3·8	7·1	983	—	13·6	—	2·7	6·5	841	—	13·6	—	2·4	6·9	536
October	—	6·5	—	2·4	3·4	1044	—	5·4	—	1·9	3·2	962	—	4·2	—	1·7	2·2	787
November	—	1·0	—	1·1	0·0	1313	—	1·0	—	1·1	0·0	1313	—	1·1	—	1·2	0·0	1192
December	—	0·9	6·8	—	0·6	1067	—	0·9	6·8	—	0·6	1067	—	1·0	6·9	—	0·6	1052
1950 January	—	0·6	6·8	—	0·2	1329	—	0·6	6·8	—	0·2	1329	—	0·6	6·9	—	0·2	1306
February	—	1·7	6·0	—	0·5	1257	—	1·7	6·0	—	0·5	1257	—	1·7	6·1	—	0·5	1253
March	—	1·8	8·7	—	1·5	1435	—	1·8	8·7	—	1·5	1435	—	1·8	8·8	—	1·5	1424
April	—	0·8	5·8	—	0·6	1045	—	0·8	5·8	—	0·6	1044	—	0·8	5·9	—	0·6	1033
May to November	—	9·5	—	3·0	5·2	6767	—	8·3	—	2·2	4·5	6077	—	6·3	—	1·7	3·5	4407
December to April	—	1·2	6·9	—	0·7	6133	—	1·2	6·9	—	0·7	6132	—	1·2	7·0	—	0·7	6068
Samples D. Heat-treated milk distributed by other than processing dairy																		
1949 May	—	4·5	—	1·2	4·1	246	—	4·6	—	0·8	3·8	240	—	4·1	—	1·0	4·1	194
June	—	28·0	—	9·1	18·9	143	—	26·2	—	8·4	18·7	107	—	26·9	—	7·7	23·1	52
July	—	28·4	—	8·6	21·6	162	—	25·6	—	4·7	19·4	129	—	25·0	—	3·6	19·0	84
August	—	36·3	—	11·1	20·7	135	—	28·7	—	7·4	12·8	94	—	31·9	—	6·4	17·0	47
September	—	28·0	—	9·0	17·5	200	—	25·9	—	6·0	14·5	166	—	19·8	—	2·5	9·9	81
October	—	17·5	—	2·6	8·7	229	—	15·9	—	2·7	0·9	220	—	14·6	—	2·7	8·6	185
November	—	0·4	—	0·0	0·8	254	—	0·4	—	0·0	0·8	254	—	0·4	—	0·0	0·8	254
December	—	0·7	13·3	—	2·3	300	—	0·7	13·3	—	2·3	300	—	0·3	12·4	—	2·1	291
1950 January	—	0·6	12·9	—	1·8	326	—	0·6	12·9	—	1·8	326	—	0·7	12·4	—	2·0	306
February	—	0·0	7·0	—	0·4	285	—	0·0	7·0	—	0·4	285	—	0·0	7·0	—	0·4	285
March	—	1·4	14·7	—	4·1	442	—	1·4	14·7	—	4·1	442	—	1·4	14·7	—	4·1	442
April	—	0·0	2·7	—	0·0	184	—	0·0	2·7	—	0·0	184	—	0·0	2·8	—	0·0	179
May to November	—	17·8	—	5·0	11·5	1369	—	14·7	—	3·3	9·3	1210	—	11·4	—	2·1	7·8	897
December to April	—	0·7	11·2	—	2·1	1537	—	0·7	11·2	—	2·1	1537	—	0·6	10·9	—	2·1	1503

The C type distributors retailing their own heat-treated milk had the lowest incidence of failures with each of the tests; while the D type distributors retailing heat-treated milk processed by other dairies had an appreciably higher incidence of failures. A and B distributors, retailing their own designated raw milk or that purchased from farms, had a rather higher incidence of failures than the D type distributors.

During the summer period, May to November, the C.O.B. test (O) after storage of samples at shade temperature was the easiest to pass, followed in order of increasing difficulty by the C.O.B. test (P) after storage at $20 \pm 1°$ C., the methylene-blue ($\frac{1}{2}$ hr.) test (M) after storage at shade temperature, and (for the designated raw milk only) by the methylene-blue ($4\frac{1}{2}/5\frac{1}{2}$ hr.) test (L).

During the winter period, December to April, the incidence of failures with all the tests was much less than in summer, and was lowest, and of the same order, with tests M and P. The methylene-blue ($\frac{1}{2}$ hr.) test (N), after storage of the samples at 20° C., proved to be more difficult to pass than the methylene-blue ($\frac{1}{2}$ hr.) test (M) after storage at shade temperature. For designated raw milk, distributors A and B, the total percentage failures with the methylene-blue tests L and N during the winter period were approximately equal, although there were differences between the individual monthly results for the two tests.

The incidence of failures with individual group C and D distributors

As stated earlier, a number of group C and D distributors was selected at random for sampling by N.A.A.S. and N.M.T.S. laboratories at approximately fortnightly intervals throughout the year. These results, and those from the commercial dairies, have been analysed separately, and show that individual distributors are able to reach the standard required for each test. The results for some C dairies are given in Table 5, and for some D dairies in Table 6. Only dairies with ten or more samples in summer, May to November, are included: winter, December to April, results for the same dairies are given when at least seven samples were tested.

In the routine control of milk the main concern is the ability or otherwise of individual distributors rather than a group of distributors to conform to prescribed standards. It may be seen from Tables 5 and 6 that some dairies had a very good record, whereas that of others was very bad. Indeed, it is obvious from these tables that the total percentage failures from a group of dairies give an entirely wrong impression of the ability of individual dairies to comply with prescribed standards; and further that the severity of any set standard must be judged by results from individual dairies and not by those from a group of dairies.

It is of interest to see how the total samples failing each test can be assigned to the distributors with good records and to those with bad ones. To this end, the results given in Tables 5 and 6 for C and D dairies tested at N.A.A.S. and N.M.T.S. laboratories were studied. The percentage of the total failures from all the distributors contributed by the 25% best and 25% worst dairies of each type are given in Table 7. A very high proportion of the total failures for both C and D dairies can be ascribed to the 25% bad records.

A record of the source of supplies distributed by group D dairies tested at N.A.A.S. and N.M.T.S. laboratories was available. Because of this, in a few cases comparison is possible of type D dairy with its type C supplier when both were tested, and in others between different type D dairies receiving supplies from the same source even though the processing dairy was not itself selected for sampling. All the appropriate results are given in Table 8.

It may be seen that some of the group D dairies had a higher incidence of test failures than the C dairies supplying them, and further, that there was considerable variation in the percentage failures from type D dairies receiving supplies from the same source. The results from group D dairies (114, 116, 129, 135) receiving milk from one and the same supplier illustrate this point well.

Table 5. *The incidence of failures in summer and winter for samples of heat-treated milk from individual Group C dairies, when maximum atmospheric shade temperature is* 75° F. *or less*

	Percentage failures							
	Summer*				Winter*			
Dairy no.	M	O	P	Total samples	M	N	P	Total samples
Samples from commercial dairies tested in P.A.B. laboratories								
1	0·0	0·0	0·0	13	0·0	0·0	0·0	9
5	0·0	0·0	0·0	13	0·0	0·0	0·0	7
2	0·0	0·0	0·0	12	0·0	0·0	0·0	10
3	0·0	0·0	0·0	12	0·0	0·0	0·0	10
4	0·0	0·0	0·0	12	0·0	0·0	0·0	9
12	0·0	0·0	0·0	12	—	—	—	†
6	0·0	0·0	0·0	12	—	—	—	†
9	0·0	0·0	0·0	11	0·0	0·0	0·0	10
21	0·0	0·0	0·0	11	0·0	0·0	0·0	10
11	0·0	0·0	0·0	11	0·0	0·0	0·0	8
16	6·7	0·0	6·7	15	—	—	—	†
10	7·1	0·0	0·0	14	0·0	0·0	0·0	9
17	7·7	7·7	7·7	13	0·0	0·0	0·0	10
14	8·3	0·0	0·0	12	0·0	0·0	0·0	9
18	8·3	0·0	16·7	12	0·0	0·0	0·0	10
15	14·3	0·0	0·0	14	0·0	0·0	0·0	8
31	16·7	0·0	25·0	12	0·0	40·0	0·0	10
32	18·2	0·0	9·1	11	0·0	87·5	12·5	8
19	20·0	0·0	0·0	10	0·0	12·5	0·0	8
23	20·0	0·0	10·0	10	0·0	0·0	0·0	8
22	23·1	0·0	7·7	13	—	—	—	†
26	25·0	0·0	25·0	12	0·0	20·0	0·0	10
28	27·3	0·0	9·1	11	0·0	20·0	0·0	10
29	27·3	0·0	27·3	11	0·0	10·0	0·0	10
25	30·0	10·0	20·0	10	—	—	—	†
24	33·3	0·0	16·7	12	0·0	0·0	0·0	9
27	45·5	0·0	9·1	11	—	—	—	†
30	45·5	9·1	36·4	11	0·0	0·0	0·0	10
35	53·8	30·8	30·8	13	0·0	50·0	20·0	10
33	54·5	9·1	27·3	11	0·0	57·1	14·3	7
34	57·1	35·7	50·0	14	0·0	0·0	0·0	10
36	63·6	18·2	27·3	11	12·5	87·5	25·0	8
Total	18·8	3·9	11·3	382	0·4	13·9	2·5	237
Samples from commercial dairies tested in their own laboratories								
01	0·0	0·0	0·0	18	0·0	0·0	0·0	18
02	0·0	0·0	0·0	92	0·0	0·0	0·0	148
06	1·5	0·6	0·6	336	—	—	—	†
05	2·3	2·3	4·6	87	0·0	0·0	0·0	141
04	3·9	0·5	2·3	560	0·0	0·2	0·0	531
03	34·8	30·4	12·7	158	26·0	76·0	5·5	200
Total	6·7	4·4	3·1	1251	5·0	14·7	1·1	1038
Grand total	9·6	4·3	5·0	1633	4·2	14·6	1·3	1275

* Summer: May to November 1949. Winter: December 1949 to April 1950.

† Less than seven samples.

Four dairies omitted entirely because less than ten samples were available in summer and less than seven in winter.

Table 6. *The incidence of failures in summer and winter for samples of heat-treated milk from individual Group D dairies, when maximum atmospheric shade temperature is 75° F. or less*

Samples from commercial dairies tested in P.A.B. laboratories

	Percentage failures							
	Summer*				Winter*			
Dairy no.	M	O	P	Total samples	M	N	P	Total samples
101	0·0	0·0	6·7	15	—	—	—	†
103	0·0	0·0	0·0	10	0·0	0·0	0·0	8
113	7·1	7·1	7·1	14	0·0	0·0	0·0	10
108	8·3	0·0	8·3	12	0·0	0·0	0·0	10
109	8·3	0·0	8·3	12	0·0	0·0	0·0	10
110	9·1	0·0	0·0	11	0·0	0·0	0·0	8
111	9·1	0·0	9·1	11	0·0	0·0	0·0	10
114	9·1	9·1	9·1	11	0·0	0·0	0·0	8
112	10·0	0·0	0·0	10	0·0	10·0	0·0	10
118	14·3	7·1	21·4	14	0·0	0·0	0·0	10
123	16·7	0·0	8·3	12	0·0	0·0	0·0	10
122	16·7	0·0	8·3	12	0·0	0·0	0·0	9
116	16·7	8·3	8·3	12	0·0	0·0	0·0	8
124	18·2	0·0	9·1	11	0·0	0·0	0·0	8
121	18·2	9·1	18·2	11	0·0	10·0	0·0	10
119	18·2	18·2	18·2	11	0·0	10·0	0·0	10
120	20·0	10·0	30·0	10	0·0	0·0	0·0	9
126	27·3	0·0	0·0	11	—	—	—	†
125	27·3	27·3	27·3	11	0·0	0·0	0·0	8
129	33·3	8·3	25·0	12	0·0	0·0	0·0	8
130	33·3	25·0	41·7	12	0·0	10·0	0·0	10
127	36·4	0·0	0·0	11	—	—	—	†
132	36·4	0·0	18·2	11	0·0	80·0	40·0	10
128	40·0	0·0	10·0	10	—	—	—	†
131	40·0	10·0	40·0	10	—	—	—	†
135	41·7	0·0	25·0	12	0·0	12·5	0·0	8
133	45·5	0·0	27·3	11	0·0	40·0	10·0	10
136	60·0	0·0	10·0	10	—	—	—	†
Total	21·6	5·0	14·1	320	0·0	8·4	2·5	202

* Summer: May to November 1949. Winter: December 1949 to April 1950.

† Less than seven samples.

Eight dairies omitted entirely because less than ten samples were available in summer and less than seven in winter.

Table 7. *The proportion of the total failures attributable to 25% good dairies and 25% bad dairies*

		Percentage of total failures attributable to good and bad dairies					
		Summer*			Winter*		
	No. of dairies	M	O	P	M	N	P
		Group C. Milk delivered by processing dairy					
Good dairies	8	0·0	0·0	0·0	0·0	0·0	0·0
Bad dairies	8	61·2	86·7	62·8	100·0	60·6	83·3
Total no. of failures from thirty-two dairies		72	15	43	1	33	6
		Group D. Milk delivered by other than processing dairy					
Good dairies	7	7·2	6·3	11·1	0·0	0·0	0·0
Bad dairies	7	44·9	43·8	46·6	0·0	76·5	100·0
Total no. of failures from twenty-eight dairies		69	16	45	0	17	5

* Summer: May to November 1949. Winter: December 1949 to April 1950.

Table 8. *Results for group D dairies receiving milk from the same source*

	Dairy no.	Percentage failures: Summer* M	Summer* O	Summer* P	Summer* Total samples	Winter* M	Winter* N	Winter* P	Winter* Total samples	Delivered in bottles or in bulk
D distributor	108	8·3	0·0	8·3	12	0·0	0·0	0·0	10	Unknown
D distributor	109	8·3	0·0	8·3	12	0·0	0·0	0·0	10	Unknown
C supplier	31	16·7	0·0	25·0	12	0·0	40·0	0·0	10	—
D distributor	111	9·1	0·0	9·1	11	0·0	0·0	0·0	10	Bulk
D distributor	132	36·4	0·0	18·2	11	0·0	80·0	40·0	10	Bottled‡
C supplier	9	0·0	0·0	0·0	11	0·0	0·0	0·0	10	—
D distributor	110	9·1	0·0	0·0	11	0·0	0·0	0·0	8	Both
C supplier	11	0·0	0·0	0·0	11	0·0	0·0	0·0	8	—
D distributor	136	60·0	0·0	10·0	10	—	—	—	†	Bulk
C supplier	6	0·0	0·0	0·0	12	—	—	—	†	—
D distributor	114	9·1	9·1	9·1	11	0·0	0·0	0·0	8	Bottled
D distributor	116	16·7	8·3	8·3	12	0·0	0·0	0·0	8	Bottled
D distributor	129	33·3	8·3	25·0	12	0·0	0·0	0·0	8	Bottled
D distributor	135	41·7	0·0	25·0	12	0·0	12·5	0·0	8	Bottled
D distributor	126	27·3	0·0	0·0	11	—	—	—	†	Bulk
D distributor	127	36·4	0·0	0·0	11	—	—	—	†	Bulk
D distributor	121	18·2	9·1	18·2	11	0·0	10·0	0·0	10	Bulk
D distributor	133	45·5	0·0	27·3	11	0·0	40·0	10·0	10	Bulk

* Summer: May to November 1949. Winter: December 1949 to April 1950.

† Less than seven samples.

‡ Milk occasionally supplied in bulk, but mainly bottled.

DISCUSSION

In general, dairies distributing heat-treated milk had a lower incidence of failures with each of the tests than those distributing raw milk. Further, dairies distributing their own heat-treated milk had better results than those retailing milk processed at a different dairy.

A study of the results for individual dairies distributing heat-treated milk, and sampled regularly throughout the year, showed that some were consistently able to pass each of the tests, whereas others frequently failed. If, as has been suggested by Wilson (6), compliance with the standard for 75% of the samples tested during a given period is accepted as the basis of judgement, the majority of these dairies would be classed as satisfactory. Using this criterion, consideration of the results in winter and summer separately is likely to give more information about the quality of milk distributed by a particular dairy, as the tendency for a low incidence of failures in winter may unduly weight the results for the year in favour of the distributor.

In summer, the standard of the C.O.B. (shade storage) test (O) for heat-treated milks proved the most lenient, and that of the methylene-blue ($\frac{1}{2}$ hr.) (shade storage) test (M) the most severe; the standard of the C.O.B. (20° C. storage) test (P) occupied an intermediate position. For the designated raw milks the standard of the methylene-blue ($4\frac{1}{2}/5\frac{1}{2}$ hr.) test (L) was approximately the same as that of the methylene-blue ($\frac{1}{2}$ hr.) (shade storage) test (M), although there were occasional wide differences between the results of these two tests.

In winter, the incidence of failures with the C.O.B. (20° C. storage) test (P) usually approximated to that for the methylene-blue ($\frac{1}{2}$ hr.) (shade storage) test (M) with both

raw and heat-treated milks; with the methylene-blue (½ hr.) (20° C. storage) test (N) and the methylene-blue (4½/5½ hr.) test (L) the incidence of failures for designated raw milks was markedly higher.

At present, results for the methylene-blue (½ hr.) (shade storage) test (M), applied to samples of pasteurized milk, are void if the maximum temperature (in the cabinet used for storage of samples) for the 24 hr. period beginning at 9 a.m. on the day of sampling exceeds 65° F. The figures in Table 3 show that for the months of June to September test results on many of the samples taken for examination would be void if a bar were to be placed at 65° F. Of the 5,995 samples from the four types of distributor tested during these 4 months, 63% were examined when the temperature exceeded 65° F. Presumably a bar has been placed at 65° F. because the standard with this test is thought to be too severe for samples stored at a higher temperature. In practice, consumers are most likely to experience difficulty with milk of poor keeping quality when temperatures are high; to be informed that tests are void because the temperature exceeds 65° F. is poor consolation to an aggrieved consumer. It would seem reasonable either to modify the standard or to apply another test. Consideration of the results obtained shows that, in any one month, the incidence of failures for all samples examined by each test at maximum shade temperatures up to 70° F., or even 75° F., is not much higher than at temperatures up to 65° F. (Table 4). Moreover, many of the dairies distributing heat-treated milk (Tables 5 and 6) experienced no difficulty in reaching the standard required for the different tests for all samples examined when maximum temperatures were 75° F. or less.

The present survey was undertaken to obtain information about the suitability of different tests for the routine control of milk sampled at the time of distribution to the consumer. There is, unfortunately, no yardstick which would serve unequivocally for the evaluation of the different tests. However, the consumer, in whose interest routine control of milk is undertaken, being assured of the safety of milk is interested mainly in its keeping quality. In Britain, milk is delivered daily and the consumer expects it to keep for 24 hr. until the next delivery. The consumer's interest would therefore be served by a test which would ensure that the milk he receives will keep for a period of 24 hr. with storage at shade atmospheric temperature—the usual conditions in the consumer's house.

It is beyond doubt that milk which fails a C.O.B. test, applied after storage at shade temperature until 9–9.30 a.m. on the day after distribution, is unsatisfactory to the consumer. Indeed, milk which satisfies this test may be unsatisfactory, for milk is sometimes unpalatable several hours before the C.O.B. end-point is reached (3). This test, as applied in the present trials, cannot be relied upon to ensure that milk is always satisfactory to the consumer. To provide the margin necessary to ensure palatable milk to the consumer 24 hr. after its delivery, it may be desirable either to increase the pre-test storage period, e.g. C.O.B. test done at 12 noon instead of 9–9.30 a.m., or to increase the pre-test storage temperature, e.g. C.O.B. test applied after storage at 20° C. at 9–9.30 a.m. No information on the incidence of failures to be expected if the test were to be applied at 12 noon is available from the present survey. Continuous storage at a mean temperature as high as 20° C. will seldom occur in the consumer's house, and a C.O.B. test after storage at this temperature should, in the main, ensure palatable milk to the consumer except in hot weather. It may, of course, be argued that storage at 20° C. is artificial and that some samples which would remain sweet in the consumer's house will fail the C.O.B. test after holding at this temperature, particularly in winter. However, it is obvious from the

results in these trials that, in winter, to ensure compliance with a test for keeping quality after storage overnight at 20° C. need present no difficulty in the production, processing, handling and distribution of milk. Storage of samples at a constant temperature has the further advantage in that it largely eliminates the variable treatment accorded samples because of day-to-day variations in shade atmospheric temperatures and in this way, therefore, it is possible both to obtain more reliable information on the general quality of milk supplies from occasional samples and to accord relatively equitable treatment to all dairies in that sampling on cold or warm days has less effect on the results.

Milks which satisfy the methylene-blue (½ hr.) test (M) after storage at shade temperature are likely to be of a keeping quality adequate to satisfy normal consumer requirements. Indeed, if it is accepted that the keeping quality of samples which pass a C.O.B. test (P) after storage at 20° C. is adequate, there is evidence in the present survey that, in summer, some milks which fail the methylene-blue (½ hr.) test (M) after storage at shade temperature may be of a sufficiently good keeping quality to satisfy the consumer, since many samples failing this dye test do pass the above C.O.B. test. These remarks apply with greater force to the results of the methylene-blue (4½/5½ hr.) test (L); the incidence of failures for raw milks is, in general, higher with this than with any of the other tests.

If it is desired to select a test that can be applied throughout the year to both designated raw and heat-treated milks, the choice must be between the methylene-blue (½ hr.) test (M) after storage at shade temperature and the C.O.B. (20° C. storage) test (P). The methylene-blue (4½/5½ hr.) test (L) and the methylene-blue (½ hr.) test (N) after storage at 20° C. are both unnecessarily stringent in the winter months, and the C.O.B. test (O) after storage at shade temperature overnight is too lenient.

The methylene-blue (½ hr.) test (M) after storage at shade temperature carried out on raw milk gives results that correspond fairly closely with those of the methylene-blue (4½/5½ hr.) test (L) during the summer months but returns fewer failures in the winter. The C.O.B. test (P) (20° C. storage) consistently gives a lower incidence of failures than the methylene-blue (4½/5½ hr.) test (L) throughout the year, and lower than the methylene-blue (½ hr.) test (M) after shade storage during the summer months but approximately equal to those of the latter test in winter.

The real problem is whether the margin of safety given by the methylene-blue (½ hr.) test (M) after shade storage is too great or whether that by the C.O.B. test (P) after storage at 20° C. is too small to ensure that milk will keep sweet for 24 hr. after delivery to the consumer. In favour of the latter test is the smaller range of variation in the proportion of failures during summer and winter, but against it is the fact that it demands a lower standard of quality for both raw and heat-treated milk than the standards at present required by the official regulations. But there is no virtue in fixing standards which cannot reasonably be enforced.

Whichever of these tests is used for the examination of samples of milk taken at the time of distribution to the consumer it seems, from the evidence presented, that no material increase in the incidence of failing samples is likely to occur if all samples are tested when the maximum shade temperature is not is excess of 70° F. or even 75° F. With the present bar at 65° F.(2) the results for many samples tested during the summer months are void, and the consumer receives least protection when it is most needed.

We would take this opportunity to emphasize that a test which may serve to indicate the keeping quality of milk in the hands of the consumer may not be a suitable measure of

the efficiency of individual dairies. Moreover, when samples from a particular dairy are unsatisfactory, it may be necessary to do a series of special tests to reveal the source of defects in the methods of milk production at the farm, and its handling and processing at dairies.

SUMMARY

1. This survey was undertaken to select a test for the official examination of the keeping quality of milk, both raw and pasteurized, sampled during its distribution to the consumer. Forty-five laboratories in England and Wales collaborated in the survey and together tested 20,159 samples during the year May 1949–April 1950.

2. Failures with each test were lower in winter than in summer, and increased with increasing shade temperature. However, the proportion of failures for all samples tested in any one month at maximum shade temperatures up to 70° F., or even 75° F., was not appreciably higher than at temperatures up to 65° F. Of the samples examined during June to September, 62% were taken on days when the maximum shade temperature exceeded 65° F.

Whatever test is used for the control of milk it is reasonable to expect that at least 75% of samples taken during a given period should reach the required standard. Because of the lower incidence of failures in winter than in summer, this requirement should apply to tests done during a period of 6 months rather than one of a year.

3. In summer, the C.O.B. test (O) after storage at shade temperature gave the lowest incidence of failures, followed by the C.O.B. test (P) after storage at 20° C., the methylene-blue (½ hr.) test (M) after storage at shade temperature and, for designated raw milk, the methylene-blue (4½/5½ hr.) test (L).

In winter the methylene-blue (½ hr.) test (M) after shade storage gave approximately the same proportion of failures as the C.O.B. test (P) after storage at 20° C. Failures with the methylene-blue (½ hr.) test (N) after storage at 20° C. were much higher and, for designated raw milk samples, of the same order as those with the methylene-blue (4½/5½ hr.) test (L).

4. Dairies retailing their own heat-treated milk had the lowest percentage of failures with each of the tests. Failures were appreciably higher, especially in summer, for dairies retailing heat-treated milk purchased from other dairies; and still higher for producer-retailers and distributors of designated raw milk.

Examination of the results for individual dairies distributing heat-treated milk showed that some had no failures by any of the tests, whereas others had many. The latter dairies were responsible for a high proportion of the total failures.

5. Of the tests used in the survey, the C.O.B. test (P) after storage of samples from 4 p.m. on the day of distribution until 9 to 9.30 a.m. the following morning at 20° C. approximates most closely to the ideal required to indicate that milk delivered to consumers is of a satisfactory keeping quality. The standard with this test, in summer, is definitely more lenient than that of the methylene-blue (½ hr.) test (M) after storage at shade temperature which is, at present, used officially for the control of pasteurized milk (2), and, throughout the year, is much lower than that of the methylene-blue (4½/5½ hr.) test (L) now used for the control of designated raw milk (1). It is doubtful whether this C.O.B. test (P) would detect all milks that are likely to go bad in the consumer's household within 24 hr. of delivery. On the other hand, the evidence also shows that the methylene-blue (½ hr.) test (M) after shade storage will condemn a number of samples which would, in fact,

keep sweet in the consumer's house. If either test were to be adopted, it would be reasonable to require samples tested at a *maximum* shade temperature up to 70° F. or even 75° F. to reach the standard.

We should like to acknowledge our indebtedness to the management and laboratory staff of each of the six commercial dairies, and to those responsible for the work at the twenty-four P.H.L.S. and at the fifteen N.A.A.S. and N.M.T.S. laboratories, without whose willing collaboration this extensive survey of milk supplies throughout England and Wales could not have been undertaken.

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453. NUTRITIVE VALUE FOR RATS OF SOW'S MILK IN COMPARISON WITH COW'S MILK

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The young suckling piglet normally doubles its birth weight in 7–10 days, and frequently even sooner. This growth rate, in relation to size, is very high, greater than that of most other mammals.

The chemical composition of the milk produced by different species of animal, in terms of total protein, lactose, fat and ash, shows very wide variation, and it is known that some of the differences in composition are related to the stage of development at which the young are born and, in part, to subsequent growth.

It has long been our intention to investigate the nutritive value of sow's milk in order to determine whether it has any special property, other than high caloric value, partly or wholly responsible for this very rapid weight increase of the newborn piglet.

Because of the high degree of control exercised by the sow, it was, until quite recently, possible to obtain only very small quantities of sow's milk, and this only with great difficulty by subterfuge during natural suckling. Consequently, no large-scale work on the nutritive properties of sow's milk could be contemplated. However, in 1940 Elly & Petersen (1) devised a method for inducing the 'let-down' of milk in the cow by the injection of the oxytocic hormone from the posterior lobe of the pituitary gland. This technique was successfully applied to the sow by Braude, Coates, Henry, Kon, Rowland, Thompson & Walker (2) who obtained in this way considerable quantities of milk by hand milking. The collection of the large quantities of sow's milk required for feeding experiments was thus made possible.

So far as we are aware, very little laboratory work on the nutritive properties of sow's milk has, up to now, been done, and that with small numbers of experimental animals, no doubt owing to the difficulty of obtaining the necessary supplies of the milk. Carpenter (3) gave mineralized sow's milk without stint to six rats, and compared their growth rate with that of six rats given similarly mineralized cow's milk. The average gain in weight of the rats given sow's milk was 140 g. over a period of 4 weeks, whereas the rats given cow's milk gained 121 g. Whitehair, Grummer, Phillips, Bohstedt & McNutt (4), in work on gastroenteritis in baby pigs, compared the growth rate of three rats fed on sow's milk with that of three rats fed on cow's milk. The milks were again given without stint but were not mineralized. The average gains over 4 weeks were respectively 72 and 36 g. Heidebrecht, Ross, Whitehair & MacVicar (5), again in work on disease in baby pigs, obtained an average gain in 4 weeks of 122·8 g. with four rats given mineralized sow's milk without stint compared with 121·2 g. with four rats fed on Jersey milk. The sow's milk used was obtained from sows whose litters showed symptoms of baby-pig disease. With milk from a sow with a healthy litter an average gain of 114·0 g. with three rats was obtained.

EXPERIMENTAL

Plan of experiments

This paper describes four experiments that we have carried out on the nutritive value of sow's milk for the rat in comparison with that of cow's milk.

As the percentage of total solids in sow's milk is much higher than in cow's milk, there is a correspondingly large difference in the caloric value of the two milks. Since we wished to determine whether sow's milk had any special property, other than its high caloric value, responsible for the rapid growth of suckling piglets, in our experiments the cow's and sow's milks were fed on a comparable basis of caloric value. In the first and third experiments, the sow's milk was diluted and compared with normal cow's milk, and in the second experiment, evaporated cow's milk was diluted and compared with undiluted sow's milk. In the fourth experiment in which the biological value of the proteins of cow's and sow's milks were compared, both milks were freeze dried and fed in amounts which supplied about 8% of protein in the diets.

Exp. 1

Rats. Twelve pairs of male litter-mate rats, 3 weeks old at the commencement of the experiment, were used. One rat from each pair was chosen by lot to receive cow's milk, while the other received diluted sow's milk.

Cow's milk. The cow's milk used was obtained from the Institute herd of Guernseys, the total requirement for the experiment of about 6 gal. being obtained from an afternoon milking and the following morning milking. The milk was bulked, kept at a temperature of $-25°$ for 12 hr., thawed and finally put into cartons, each holding about 300 ml. These cartons were stored at $-25°$ until required for feeding. During the experiment the estimated daily quantity of milk needed was thawed out overnight at room temperature.

Sow's milk. The sow's milk was collected from the Institute and neighbouring herds of Large White sows over a period of a month. On collection the milk was stored in cartons at $-25°$. When the total quantity of about 4 gal. required for the experiment had been collected, it was thawed, bulked and re-stored in cartons at $-25°$ in the manner described for the cow's milk.

To determine the caloric value of the two milks, the formula of Overman & Sanmann (6)

$$\text{cal./quart}^* = 90{\cdot}67 \times \text{fat}\,\% + 54{\cdot}27 \times \text{protein}\,\% + 26{\cdot}73 \times \text{lactose}\,\% + 55{\cdot}44$$

was employed, using the analyses given in Table 1. For this experiment, the estimated quantity of sow's milk required was thawed out daily at room temperature, and 22·9 ml. of water were added to each 77·1 ml. of the milk before feeding.

The calculated composition of the diluted sow's milk is given in Table 1, and the caloric values of the cow's and sow's milks as fed, obtained by application of the above formula were 914·8 and 934·8 cal./quart respectively. This difference arose since the density of the two milks had not been taken into account when calculating the dilution factor. However, in assessing the significance of the results, the difference in caloric value was allowed for.

The mineral supplement as used by Elvehjem, Hart, Jackson & Weckel (7) was given to each rat daily in the milk. The rats were housed in separate cages and their general management was as described by Henry & Kon (8).

The paired feeding technique of equalizing intake of milk was used. The experiment lasted for 4 weeks.

* 1 quart (American) = 946·36 ml.

Table 1. *Chemical composition of the cow's and sow's milks used in Exp.* 1 (*percentage by weight*)

Constituent	Cow's milk	Sow's milk	
		Before dilution	After dilution (calculated)
Total solids	14·74	19·27	14·98
Fat	5·88	7·60	5·91
Solids-not-fat	8·86	11·67	9·07
Protein	3·92	5·62	4·37
Lactose	4·25	5·12	3·98
Ash	0·69	0·93	0·72

Exp. 2

Twelve pairs of 3-weeks-old litter-mate male rats were again used. One rat from each pair received diluted evaporated cow's milk and the other received sow's milk.

The 4 gal. of sow's milk required for this experiment were collected over a period of 1 month and stored as described in Exp. 1. About half the sow's milk for this experiment came from Wessex Saddleback sows, the rest being obtained from Large Whites. The full cream, unsweetened evaporated milk required for this experiment was obtained in 16 oz. tins from Wilts United Dairies Ltd. The consignment was all from one bulk and of uniform quality.

Table 2. *Chemical composition of the cow's and sow's milks used in Exp.* 2 (*percentage by weight*)

Constituent	Evaporated cow's milk		Sow's milk
	As received	After dilution (calculated)	
Total solids	31·60	22·13	19·02
Fat	9·20	6·44	6·90
Solids-not-fat	22·40	15·69	12·12
Protein	8·73	6·11	6·09
Lactose	11·65	8·16	5·09
Ash	2·02	1·42	0·94

The evaporated milk was stored at room temperature in the sealed tins and was diluted daily as required. The formula used in Exp. 1 was applied to determine the caloric value of the two milks; 31·5 ml. of water were added to each 68·5 ml. of evaporated milk before feeding.

The calculated composition of the diluted evaporated milk is given in Table 2, and the caloric value of the cow's and sow's milks as fed, obtained by application of Overman & Sanmann's formula, was 1189·1 and 1147·3 cal./quart respectively. As in Exp. 1, this difference in the caloric value of the two milks was taken into account in assessing the significance of the results.

The same mineral supplement as used in Exp. 1 was given to all rats daily, and the general management was as previously described. The experiment lasted for 26 days.

Exp. 3

Twelve litters of four 3-weeks-old litter-mate male rats were used. One animal from each litter received at random one of the following four treatments:

1	2	3	4
Sow's milk diluted to same caloric value as cow's milk	Cow's milk	As treatment 1 with synthetic vitamin supplement*	As treatment 2 with synthetic vitamin supplement*

* See Table 4.

All milks in this experiment were given without stint. The Guernsey cow's milk used was obtained and stored as previously described for Exp. 1.

About 8 gal. of sow's milk required for this experiment were collected from Large White and Wessex Saddleback herds of sows over a period of about 10 weeks. The milk was stored at −25° and later bulked and re-stored in 300 ml. cartons ready for use in the manner described in Exp. 1. In order to make a more accurate determination of the caloric value of the two milks than that given by the formula used in Exps. 1 and 2, the density of the bulked sow's and cow's milks was determined and the formula of Overman & Sanmann (6),

$$\text{cal./quart (American)} = 52{\cdot}78 \times \text{fat}\,\% + 16{\cdot}41 \times \text{protein}\,\% + 37{\cdot}87 \times \text{total solids}\,\% + 46{\cdot}91 \times \text{density} - 2{\cdot}75 \text{ lactose}\,\% - 57{\cdot}70$$

was used. From figures given in Table 3, it was found that 69·5 ml. of sow's milk contained about the same number of calories as 100 ml. of the cow's milk; 30·5 ml. of water were

Table 3. *Chemical composition of the cow's and sow's milk used in Exp.* 3 (*percentage by weight*)

Constituent	Cow's milk	Sow's milk: Before dilution	Sow's milk: After dilution (calculated)
Total solids	13·64	18·86	13·25
Fat	4·75	6·98	4·90
Solids-not-fat	8·89	11·88	8·35
Protein	3·38	5·68	3·99
Lactose	4·79	5·27	3·70
Ash	0·720	0·928	0·652
Density at 20°	1·0284	1·0372	1·0259

Table 4. *Composition of synthetic vitamin supplement used in Exp.* 3

Vitamin	Amount/0·5 ml.
Aneurin	30 μg.
Riboflavin	50 μg.
Pyridoxin	30 μg.
Calcium pantothenate	100 μg.
Folic acid	5 μg.
Biotin	5 μg.
p-Aminobenzoic acid	100 μg.
Nicotinic acid	100 μg.
Inositol	1 mg.
Choline-hydrochloride	17·3 mg.
Vitamin K	20 μg.
Vitamin B_{12}	0·2 μg.

therefore added to every 69·5 ml. of sow's milk before feeding. Table 3 shows the calculated composition of the diluted sow's milk. The caloric value of the two milks as fed was 800·1 and 806·1 cal./quart for the cow's and sow's milk respectively.

All rats were given the mineral supplement as previously used, but in addition the rats on treatments 3 and 4 each received, in the milk, 0·5 ml./day of a synthetic vitamin supplement, as described by Campbell & Kosterlitz (9), but with the addition of vitamin B_{12}. The composition of this vitamin supplement is given in Table 4.

All other details of general management of the rats were as previously described. The experiment lasted for 28 days.

Exp. 4

The biological value and true digestibility of the proteins of freeze-dried samples of whole cow's and sow's milk were determined on rats, at an 8% level of protein intake

(N × 6·38), by the method of Mitchell (Mitchell (10); Mitchell & Carman (11)). Six pairs of litter-mate female rats, 23–25 days old, were used. Details of the experimental technique are described by Henry, Kon & Watson (12). The composition and analysis of the diets used are given in Table 5.

Table 5. *Composition (per cent.) and analysis of the diets used in Exp.* 4

Component	Cow's milk diet	Sow's milk diet
Freeze-dried cow's milk	32·9	—
Freeze-dried sow's milk	—	24·4
Salts (de Loureiro (13))	4·0	4·0
Margarine fat	0·3	2·2
Sugar, granulated, ground	12·0	12·0
Potato starch	10·0	10·0
Rice starch	40·8	47·4
% N	1·212	1·221
% moisture	8·81	9·29

RESULTS

Exp. 1

Table 6 gives the relevant data. The difference in the caloric value of the two milks as fed was taken into account in determining the figures for calories consumed/g. live-weight gain. It is seen that the rats that received the sow's milk grew at a significantly greater rate during the 28 days on experiment than those given cow's milk. In addition, the rats receiving the sow's milk consumed significantly less calories/g. live-weight gain than those given cow's milk.

Table 6. *Mean live-weight gain and food utilization of rats in Exp.* 1

Type of milk	No. of rats	Initial weight (g.)	Final weight (g.)	Total gain (g.)*	Total milk intake (ml.)	Milk consumed/g. live-weight gain (ml.)	Calories consumed/g. live-weight gain (cal.)*
Diluted sow's	12	55·0	155·0	100·0 (±2·33)	1331·3	13·34	13·18 (±0·19)
Cow's	12	54·9	143·6	88·7 (±3·62)	1331·3	15·28	14·77 (±0·55)
†				5·711			3·152

* Values with their standard errors.

† The appropriate significance levels of t for 11 degrees of freedom are: 1%, 3·106; 0·1%, 4·437.

Exp. 2

The relevant data are given in Table 7. In this experiment there was no significant difference in growth rate between rats receiving sow's milk and diluted evaporated cow's milk, but the appreciable difference in the caloric value of the two milks as fed invalidated a comparison of live-weight gain. When this difference in the caloric value was taken into account and the calories consumed/g. live-weight gain were determined, it was found that the rats receiving sow's milk consumed significantly less cal./g. live-weight gain than those rats given the evaporated cow's milk.

Two factors which may have some bearing on the lower significance levels obtained in this experiment as compared with Exp. 1 should be mentioned.

In Table 8 the total number of times that a measurable quantity of milk was left after 24 hr. and the total quantity refused during the course of the two experiments is shown.

These figures indicate that, whereas in Exp. 1 the rats receiving sow's milk were not limited to any appreciable extent in the amount of milk offered to them as a result of the refusals by the rats receiving cow's milk, in Exp. 2 the former were being limited in their milk intake as a result of the greater number of refusals by the rats receiving the diluted evaporated milk.

Table 7. *Mean live-weight gain and food utilization of rats in Exp.* 2

Type of milk	No. of rats	Initial weight (g.)	Final weight (g.)	Total gain (g.)*	Total milk intake (ml.)	Milk consumed/g. live-weight gain (ml.)	Calories consumed/g. live-weight gain (cal.)*
Sow's	12	53·5	151·1	97·6 (±6·96)	1123·8	11·54	13·99 (±0·22)
Diluted evaporated cow's	12	53·8	150·9	97·2 (±9·56)	1122·3	11·62	14·61 (±0·38)
t†				0·290			2·880

* Values with their standard errors.

† The appropriate significance levels of *t* for 11 degrees of freedom are: 5%, 2·201; 2%, 2·718; 1%, 3·106.

Table 8. *Refusals of milk by the rats in Exps* 1 *and* 2

Exp. 1				Exp. 2			
Diluted sow's milk		Cow's milk		Sow's milk		Diluted evaporated cow's milk	
Total no. of times that milk was refused	Total amount of milk refused (ml.)	Total no. of times that milk was refused	Total amount of milk refused (ml.)	Total no. of times that milk was refused	Total amount of milk refused (ml.)	Total no. of times that milk was refused	Total amount of milk refused (ml.)
132	506·0	159	649·0	89	181·5	200	601·5

A second factor that might have temporarily limited the intake of sow's milk was that, for 6 days during the course of Exp. 2, some of the sow's milk left over in the morning was found to be sour. A third feed at 10·0 p.m. was introduced to overcome this. The souring was apparently due to some dust which got into a few of the cartons of sow's milk during storage; when these were finished after 1 week, the normal twice daily feeding was re-introduced, and no further trouble was experienced.

Exp. 3

The relevant data are given in Table 9. The results obtained in this experiment show that the rats that received sow's milk, either with or without the vitamin supplement, grew at a greater rate and utilized the energy consumed (in terms of cal. consumed/g. live-weight gain) significantly more efficiently than those rats given cow's milk either with or without the vitamin supplement. Also, it is seen that the addition of the synthetic vitamin supplement to both milks resulted in a slightly higher consumption of milk, but the rate of growth and efficiency of energy utilization were not significantly altered.

Exp. 4

The results are given in Table 10 and show that the biological value for the rat of the proteins of cow's milk was significantly higher than that of the proteins of sow's milk, but that the true digestibilities of the proteins of the two milks did not differ significantly.

Table 9. *Mean live-weight gain and food utilization of rats in Exp.* 3

Treatment	No. of rats	Initial weight (g.)	Final weight (g.)	Total gain (g.)*	Total milk intake (ml.)	Milk consumed/g. live-weight gain (ml.)	Calories consumed/g. live-weight gain (cal.)*
1. Diluted sow's milk	12	49·3	158·8	109·6 (±4·06)	1541·8	14·19	12·09 (±0·26)
2. Cow's milk	12	49·3	139·6	90·3 (±3·14)	1429·5	15·97	13·51 (±0·32)
3. Diluted sow's milk with vitamin supplement	12	49·1	164·1	115·0 (±4·16)	1638·7	14·29	12·17 (±0·20)
4. Cow's milk with vitamin supplement	12	48·8	137·7	88·8 (±4·54)	1448·6	16·61	14·05 (±0·50)
t†		Treatments 1 and 2, 3·741. Treatments 2 and 3, 3·512.		Treatments 3 and 4, 4·937. Treatments 1 and 4, 5·166.			

* Values with their standard errors.

† The appropriate significance levels of t for 33 degrees of freedom are: 1%, 2·734; 0·1%, 3·614.

Table 10. *Mean biological values and true digestibilities of the proteins of sow's and cow's milk. Mean values with their standard errors for twelve rats*

Milk	Biological value	True digestibility
Sow's	85·1 (±1·40)	93·2 (±0·96)
Cow's	90·3 (±0·79)	95·2 (±0·53)
t*	2·89	1·84

* The appropriate significance levels of t for 11 degrees of freedom are: 5%, 2·201; 2%, 2·718; 1%, 3·106.

DISCUSSION

The results of Exps. 1–3 strongly suggest the presence in sow's milk of some factor(s) or special property, other than its high caloric value, not found in cow's milk, enabling the rat to grow faster and/or to utilize the energy consumed more efficiently. This was so whether the intake of the sow's or cow's milk was equalized as in Exps. 1 and 2, or whether the milks were given without stint as in Exp. 3.

Although a significant difference in the efficiency of energy utilization was shown in Exp. 2, the difference in the caloric value of the two milks as fed invalidated, in this experiment, a comparison on the basis of live-weight gain. Other factors which might possibly have had some effect on the results obtained in this experiment were the limitation in the milk intake of the rats receiving sow's milk, and the heating and homogenization that the evaporated cow's milk underwent in the course of its commercial preparation. In future experiments that are planned it is hoped to study the problem further; the study will include an inquiry into the possible effects of the difference in size of fat globules in sow's and cow's milk.

We are unable at present to suggest the nature of the factor(s) or special property of sow's milk responsible for its superiority for the rat as compared with normal cow's milk. The results obtained in Exp. 4 show that neither the quality nor the digestibility of the proteins in sow's milk are responsible for this superiority, and the results of Exp. 3 indicate that none of the vitamins included in the supplement are associated with this difference in nutritive value of the two milks.

SUMMARY

1. Four experiments with 108 rats to compare the nutritive value of sow's milk and cow's milk are described. In Exps. 1–3 the milks were fed on a comparable basis of caloric value.

2. In the first experiment, in which the milk intake was equalized, rats receiving diluted sow's milk grew at a significantly faster rate and utilized their calorie intake more efficiently than rats receiving cow's milk.

3. In the second experiment, again with equalized milk intake, rats receiving sow's milk utilized their calorie intake significantly more efficiently than rats receiving diluted evaporated cow's milk.

4. In the third experiment in which the milks were given without stint, rats receiving diluted sow's milk either with or without the addition of a synthetic vitamin supplement, grew at a faster rate, and utilized their calorie intake significantly more efficiently than rats receiving cow's milk either with or without the same vitamin supplement. The vitamin supplement did not improve the nutritive value of either milk.

5. In the fourth experiment the biological value of the proteins in freeze-dried sow's milk (85·1) proved significantly lower than in freeze-dried cow's milk (90·1).

We wish to thank Glaxo Laboratories Ltd for kindly carrying out the freeze-drying of the cow's and sow's milk, and Dr S. J. Rowland for the analysis of the milk samples. We also gratefully acknowledge the help given by Miss E. S. Baxter, Mr W. H. Farmer, Mr C. E. Kirby and Mr J. H. B. Roy in the collection of the large quantities of sow's milk required for our experiments, by Miss M. Chapman in feeding the rats, and Miss V. Glover with the nitrogen analyses.

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454. BACTERIOLOGICAL ASPECTS OF THE REWORKING AND BLENDING OF STORED BUTTER

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(With 1 Plate)

The necessity for storing butter, either to meet winter shortages, or to build up reserves against emergencies, has raised many problems, amongst them that of a satisfactory method of distributing stored butter when it is required for consumption. Some of the difficulties that confront the butter industry of Great Britain are peculiar to this country, since most of its butter is imported, often from distant countries, and the supplies are of different qualities and types. The supplies of butter are held in deep-cold storage until required and, when released, 'prints' of suitable size and weight are prepared for distribution. The mechanical packing of butter, involving reworking of the original bulk, often accompanied by blending of butters of different types and origin, has established itself in this country and is now regarded as almost a necessity for the distribution of butter. Although there are frequent references in the literature to the fact that these processes are liable to induce renewed bacterial growth in the butter, few figures have been published which illustrate the actual effect on the flora and keeping quality of the butter so treated.

During the summer of 1949 (hot and dry) thirteen samples of 'National' butter sold in the normal way by nine retailers to their registered customers were examined bacteriologically. No information was sought as to origin and age of the butter, nor as to the facilities for storage on the premises of the retailers concerned, but undoubtedly these varied. The figures in Table 1 show that there were wide differences in bacterial count. Each sample contained yeasts, moulds, lipolytic and caseolytic bacteria (all potential spoilage agents) in varying proportions. The coliform count was generally low, probably because of the high percentages of salt.

Table 1. *Plate counts of retail butter*

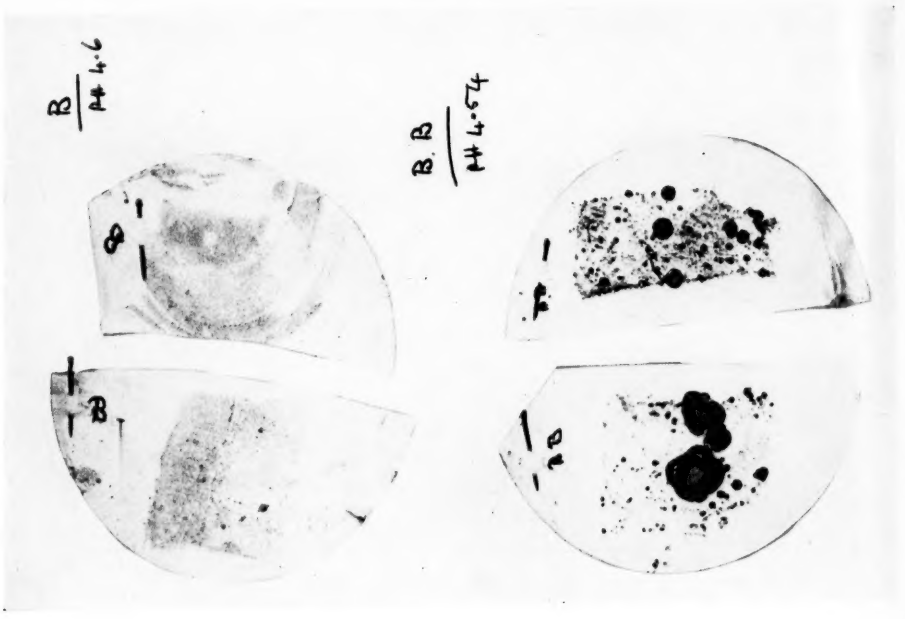
Retailer	Salt content. % in butter	Plate count/1 ml. Y.D.A.,* 30° C., 5 days	Presumptive coliform test, 30° C., 5 days (ml.)
1	2·72	2,400,000	+1/10
2	1·46	8,000	−1/10
2	2·64	76,000	−1/10
3	1·26	44,000	+1/1000
4	2·06	3,600,000	+1/10
4	1·98	1,800,000	+1/10
5	2·58	1,300,000	−1/10
6	2·44	2,400,000	−1/10
7	2·26	1,180,000	+1/100
7	2·74	2,040,000	−1/10
8	2·40	600,000	−1/10
9	2·16	2,650,000	+1/100
9	1·71	6,800,000	+1/100

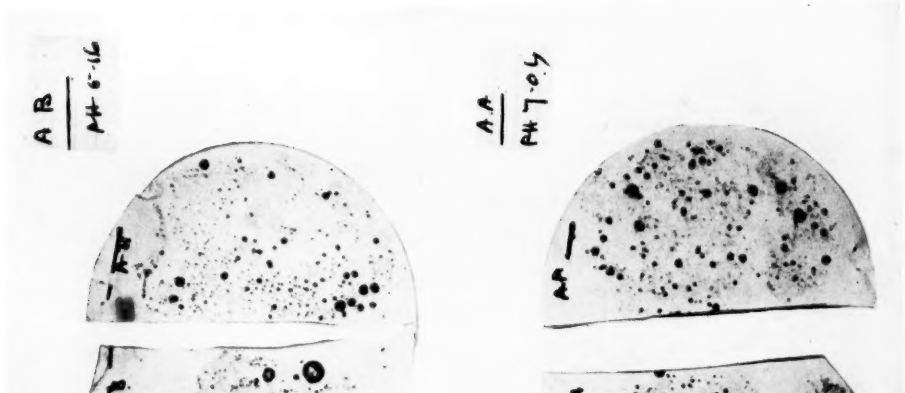
* Y.D.A. = Yeast dextrose agar.

When stored butter is reworked, not only is there almost inevitably a redistribution of bacteria in the serum droplets, many of which may hitherto have been sterile, but droplets may coalesce, offering larger supplies of nutriment and more favourable conditions for the growth of the bacteria implanted in them. Although there may be a varied flora in fresh butter many of these organisms will die under the severe conditions of several months of deep-cold storage, and the surviving organisms lying dormant in stored butter will be restricted to a comparatively few types tolerant of low temperatures and of the high salt concentration (10–12%) or high acidity (pH 4·5–4·6) in the serum of the sweet and ripened cream butters respectively; in sweet cream butter micrococci are typically the dominant organisms, yeasts in ripened cream (acid) butter. Many strains are lipolytic and proteolytic and potentially taint producers. If, as is usually the case, bacterial growth had occurred in the fresh butter, the numbers of these organisms may be higher in the stored than in the fresh butter and, when stimulated into new growth by the more favourable conditions created by reworking, may increase considerably. There is also danger of the addition of contaminants from the plant and, although some of these may be of types which will develop only poorly in butter serum, the majority may well be salt- or acid-tolerant organisms derived from other butters which have previously passed through the plant. Ripened cream (acid) butter typically contains less salt than sweet cream butter and in blends of these types the acidity and salt concentration are intermediate between those of the components. Salt is frequently added to such blends to restore the concentration to approximately the level of that of sweet cream butter, but it is not customary to readjust the acidity. It is possible that the inhibitory power of salt concentrations is influenced by variations in acidity.

The figures in Tables 2–4 indicate the reality of some of these dangers. No great change was noted in the plate counts of the butter immediately after reworking if plant contaminants were excluded—merely a redistribution of the organisms resulted from this—but on storage of this butter at either 40° F. or room temperature (R.T.) there was an increase in numbers, probably reaching a maximum at R.T. in about 14 days. The untreated butter did not usually show a significant increase.

Table 2 shows the effect of storage on samples of butter reworked and blended at a commercial plant. Four ½ lb. packets, chosen at random from each of six batches of butter, were set aside for testing. Two were tested within a few hours, the remaining pairs were held in cold store (40° F.) for 14 days. At the end of this time they were each divided into two parts, one of which was tested immediately, the other after further storage for 14 days at R.T. It is well known that the organisms are irregularly distributed in butter, and that it is difficult to gain a reliable count unless large numbers of large samples are used for each test. Such ideal conditions rarely obtain for butter, and in the tests recorded in these tables the largest possible amount (usually about ¼ lb.) was melted and well shaken, and 10 ml. of this used for the actual plate-count test. The plates were made on yeast dextrose agar, and incubated at 30° C. for 5 days. The counts were the means of duplicate plates. Bacterial growth is assumed only when the increase in plate count is well beyond the limits of experimental error. In this series of samples there was reasonable agreement between the pairs of samples immediately after the reworking, less good after storage. Even immediately after blending there was only moderate agreement between the pairs of samples of the blend of sweet cream and ripened cream butter. It is obviously more difficult to make an intimate mixture of two such dissimilar types. There was probably





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some growth of the organisms in the reworked and blended butters even at 40° F., but there was definite growth during 14 days at R.T. The increase in numbers was greatest in the samples containing acid butter.

Table 3 gives the results for other experiments on a laboratory scale in which two butters of known grade and type were reworked and blended under aseptic conditions, thus excluding plant contaminants and confining the flora to those organisms already present in the butter. A large mincer (domestic type) was used as a worker, since this could be sterilized and its action simulated that of a butter worker. The droplets of serum in the bulk butter, especially the acid butter, were generally small, but many of them were

Table 2. *Plate counts of commercial samples of reworked and blended butter*

			Plate count, Y.D.A., 30° C., 5 days			Presumptive coliform test		
				After storage			After storage	
Type of butter	Salt content (%)	Sample	Initial	Cold store 14 days	Plus R.T. 14 days	Initial (ml.)	Cold store 14 days (ml.)	Plus R.T. 14 days (ml.)
Overseas sweet cream reworked	2·57	1	40,900	—	—	+1/100	—	—
		2	37,000	—	—	+1/10	—	—
		3	—	84,000	238,000	—	+1/10	+1
		4	—	97,000	223,000	—	+1/100	+1
Overseas sweet cream reworked	2·56	1	22,700	—	—	+1/10	—	—
		2	24,100	—	—	+1/10	—	—
		3	—	84,000	990,000	—	+1	+1
		4	—	102,000	1,360,000	—	+1/10	+1
Continental ripened cream reworked	2·49	1	46,600	—	—	+1	—	—
		2	44,000	—	—	+1	—	—
		3	—	204,000	9,600,000	—	+1	+1
		4	—	172,000	4,700,000	—	+1	+1
Blend of overseas sweet cream butters of different origins	2·57	1	16,800	—	—	+1/10	—	—
		2	16,400	—	—	+1	—	—
		3	—	40,200	650,000	—	+1/10	+1
		4	—	20,000	640,000	—	+1	+1
Blend of overseas sweet cream and continental ripened cream butters	2·28	1	21,000	—	—	+1/100	—	—
		2	41,000	—	—	+1/100	—	—
		3	—	63,000	2,600,000	—	+1/100	+1/10
		4	—	58,000	3,180,000	—	+1/100	+1/100
Blend of overseas sweet cream and continental ripened cream butters	2·50	1	50,000	—	—	+1	—	—
		2	34,900	—	—	+1/10	—	—
		3	—	53,000	3,200,000	—	+1/10	+1
		4	—	72,000	3,550,000	—	+1/10	+1

much enlarged by this process (as shown by indicator papers) (Pl. 1). The overall picture was, however, little if any worse than that of ordinary retail butter.

It is interesting to note that the flora of both types of butter had apparently reached a stable phase during storage, and there was no obvious bacterial growth in either of the bulk samples during 28 days at R.T. The reworked and blended butters, on the other hand, showed definite growth. There was considerable increase in count even when these samples were held at 40° F. for 28 days. No coliform organisms were found in the acid butter, even after reworking, but obviously a few survived in the sweet cream butter and occasional samples gave positive tests. Reworking probably redistributed them more evenly in the butter, for they were found in all reworked samples, but the numbers did not increase on storage at R.T. The reworking of this sweet cream butter was repeated with another portion, using even more stringent precautions to exclude the possibility of external contamination with coliform organisms, but the result was the same, again coliform organisms were not found in the bulk sample but were detected in the reworked. The blend of sweet cream and

acid butters also contained coliform organisms, though fewer in numbers than in the reworked sweet cream sample. The serum of this butter was, however, a mixture of two types, and its acidity and salt concentration were therefore intermediate to those of its two components, and consequently less inhibitory to these organisms which increased in number during storage at R.T. In the two blends containing acid butter reported in Table 2 the coliform organisms decreased on storage, probably because of the inhibitory action of the high salt concentrations. These were commercial blends, and salt had probably been added to counteract the low salt content of the acid butter. No extra salt was added to the laboratory blends.

The importance of careful working of butter at the time of churning has frequently been pointed out in the literature on butter, and the importance of the distribution of the serum in fine droplets has been stressed. Bacteria do not, in ordinary circumstances, migrate from droplet to droplet through the fat, hence bacterial growth will be limited by the amount of nutriment available, i.e. by the size of the droplet. The finer the distribution of the

Table 3. *Plate counts of samples of butter reworked and blended in the laboratory*

	Count/1 ml. on Y.D.A., 30° C., 5 days					Coliform test and most probable numbers*						
	Held at R.T.				Held at 40° F.,	Held at R.T.				Held at 40° F.		
Sample	0 day	3 days	14 days	28 days	28 days	0 day	3 days	14 days	28 days	28 days	pH	Salt (%)
Overseas sweet cream butter:												
A Bulk	26,000	33,500	51,000	10,300	22,200	0 −1 ml.	75 +1 ml.	0 −1 ml.	0 −1 ml.	36 +1 ml.	6·94	1·51
AA Reworked	27,000	635,000	6,250,000	1,950,000	235,000	460 +1/10	240 +1/10	460 +1/10	460 +1/10	23 +1 ml.	7·04	—
Continental ripened cream (acid) butter:												
B Bulk	1,000	900	790	630	—	0 −1 ml.	0 −1 ml.	0 −1 ml.	0 −1 ml.	—	4·63	0·68
BB reworked	1,000	8,000	790,000	1,310,000	238,000	0 −1 ml.	0 −1 ml.	0 −1 ml.	0 −1 ml.	0 −1 ml.	4·54	—
AB blend of *A* and *B*	25,500	23,300	3,050,000	2,555,000	720,000	43 +1 ml.	150 +1/10	2,400 +1/100	4,300 +1/100	23 +1 ml.	5·15	1·21

* From McCrady's Tables.

moisture, the less danger there is of excessive growth of bacteria and consequently of deterioration through taints of bacterial origin. It is also well known that if butter is reworked after storage, the droplets coalesce, some of the water leaks from the butter and many of the droplets remaining in the butter are much enlarged. The fine distribution is not regained and the larger droplets offer possibilities of more extensive bacterial growth.

The data given in the tables illustrate these points, and confirm what is frequently quoted but for which few figures seem to be available in the literature. The most detailed are those of Long & Hammer (1), who made butter from cream to which cultures of several proteolytic and lipolytic organisms had been added, and tested the effect of reworking this butter after storage at 10° C. for periods up to 7 days. They found that reworking the butter enlarged the droplets, enhanced the bacterial growth and hastened the development of taint. It is obvious from the figures recorded here that reworking has a similar effect on butter which has been held in deep-cold storage for much longer periods, though here the damage may be even greater since the flora of the stored butter consists of relatively few but specialized types.

In well-made butter the extent of bacterial growth in the fine droplets is strictly limited and will probably have reached its maximum before the butter is placed in deep-cold storage. Renewed bacterial growth in such butter is unlikely even when exposed to

conditions favourable for growth, provided there is no redistribution of the organisms in the serum. Samples of bulk butter were tested before and after several days' storage at R.T. In most cases there was little increase in the bacterial count (see Table 4). Samples 1 and 6 were identical with *B* and *A* in Table 3, in which is shown the increase in count when these two samples were reworked before being placed under identical conditions of storage. The sample *AA* (Table 3) developed marked butyric rancidity within 14 days of reworking, presumably as the result of the development of the strongly lipolytic organisms which could be isolated from it.

Table 4

Type of butter	Grade	pH	Salt concentration		Plate count, Y.D.A., 30° C.		
			Butter (%)	Serum (%)	On removal from deep-cold store	After storage at R.T.	Days at R.T.
1. Continental ripened cream	1st	4·63	0·88	5·2	1,000	630	28
2. Continental ripened cream	1st	—	—	—	730,000	750,000	21
3. Continental ripened cream	Unknown	—	—	—	13,500	19,000	14
4. Continental ripened cream	Unknown	—	—	—	1,300	7,300	14
5. Continental ripened cream	—	—	—	—	5,600	26,000	14
6. Overseas sweet cream	1st	6·94	1·51	10	26,000	10,300	28
7. Overseas sweet cream	1st	—	—	—	13,000	1,160,000	21
8. Overseas sweet cream	2nd	—	—	—	199,000	1,180,000	21

Many factors combine to determine the quality and keeping quality of butter, but this paper is chiefly concerned with the bacteriological aspects. The mere numbers of bacteria (assuming the absence of pathogens) may be of minor significance; the types of organism are of greater interest. Fresh butter may contain an abundant and varied flora of which only relatively few types are potential spoilage organisms, but it is these few that are of major importance in assessing keeping quality. In stored butter, numbers are of greater importance because a higher proportion of the surviving organisms may be potential taint producers. The enhanced growth of bacteria following the reworking of stored butter, and the danger of adding contaminants from the plant, cannot be ignored in developing a procedure for the preparation of butter for retail distribution when it may be exposed to high atmospheric temperatures. It would seem that much of the deterioration in retail butter could be avoided if it were possible to eliminate the reworking of deep-cold stored butter and so limit the bacterial growth to the one short period in the fresh butter.

These studies have been confined to two types of butter, made either from sweet or from ripened cream. New problems may arise should neutralized or continuous process butters become important additions to the normal supply.

SUMMARY

1. The reworking of stored butter stimulates dormant bacteria into renewed growth and, since these are frequently taint-producing organisms, may hasten the deterioration of retail butter.

2. Blends of sweet cream and ripened cream (acid) butters may contain a greater variety of organisms than reworked samples of either type, and conditions may be created which are even more favourable for bacterial activity.

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455. THE RIPENING OF STILTON CHEESE: CHANGES IN THE VOLATILE ACID CONTENT

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(With 1 Figure)

Early studies of the ripening of Stilton cheese had indicated that significant changes in the distribution of volatile acids occurred with the growth of the mould. It also appeared probable that the extent of these changes was influenced by pricking, a practice which is widely adopted by the industry as a means of eliminating loss by non-blueing and of hastening the ripening process.

A fuller knowledge of these and other changes initiated by mould activity seemed important to the understanding of the ripening process of Stilton cheese. Special attention was paid to the volatile fatty acids, and the method previously described (1) was used for their extraction and estimation.

It was planned to include in the experiment several batches of cheese, both pricked and unpricked, in order to compensate for unavoidable variations in the manufacture and ripening of this type of cheese. The outbreak of war intervened, however, and since post-war conditions made it difficult to resume these experiments as originally planned, it was thought advisable to offer the results as they stand and report the findings for four batches of cheese. The cheese were made in the normal way from market milk of average quality, but one batch was lightly pricked 2 months after manufacture. In addition, two cheese, IS_x from this batch and $IIIS_x$ from another, were heavily pricked at 2 months and again 2 weeks later. Apart from this, the cheese were ripened under the same conditions. They were examined at pre-arranged intervals, one cheese, taken at random, being used for each examination. Bacteriological examination by means of platings and sections (2), chemical analyses of a complete quarter and colour photographic records were made for each. Some of the relevant analytical data are given in Table 1 and in Fig. 1. The protein breakdown was determined by Davies's method (3), the pH by the quinhydrone electrode and the volatile acids by direct steam distillation and the extraction method (1). For samples IS_4 to IS_9, $IIIS_4$ to $IIIS_{12}$ and IVS_3 to IVS_{12} inclusive, the amounts of CO_2 in the distillates were estimated and the totals suitably adjusted. The earlier totals were adjusted by subtracting quantities of CO_2 estimated from the results for VS. The degree of blueing was the averaged estimates computed by four observers from colour photographs and the notes made on the condition of the cheese at the time of cutting.

Cheeses IS_1 to IS_9 represent a batch of Stiltons, from one bulk of milk, which were lightly pricked with stainless steel wires of s.w.g. 16 on the 63rd day after manufacture. Mould growth was observed in each of these cheese when trial borings were taken 4 days later, and it was well established in IS_4 when this cheese was cut for analysis on the 76th day, i.e. 13 days after pricking. By the 90th day, i.e. 27 days after pricking, the growth

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Table 1

Cheese code no.		Age (days)	Moisture (%)	Protein breakdown (% total nitrogen) NP	NPS	NH_4	Acidity pH	Volatile acids, ml. N/10 acid/100 g. Direct steam dist.	Extraction method Total	H_2O fract.	Fat fract.	H_2O insol.	Degree of ripeness Blueing	CO_2 in curd
							Pricked							
1	IS_1	1	60·9	—	—	—	4·95	8·03	(25·00	12·3	15·7)	—	0	—
	IS_2	34	38·9	11·5	3·8	0·83	4·46	16·54	(32·55	18·45	13·7)	—	0	—
	IS_3	62	34·58	10·8	5·0	0·83	4·30	19·9	(29·12	21·25	7·87)	—	0	—
2	IS_4	76	32·99	15·1	6·5	1·91	4·73	12·25	21·15	10·15	11·00	—	20	15·00
	IS_5	90	30·55	19·29	7·10	3·08	5·05	11·6	38·66	12·60	26·06	15·45	50	11·50
	IS_6	104	28·85	21·6	8·4	3·46	5·16	10·32	29·25	11·25	18·00	12·60	60	15·50
3	IS_7	118	27·91	25·97	10·07	4·22	4·99	10·75	26·40	8·5	17·9	12·10	50	6·00
	IS_8	132	28·85	34·3	12·5	6·4	5·81	9·75	27·95	10·00	17·95	14·90	50	8·25
	IS_9	147	27·38	31·88	12·5	6·47	5·72	11·63	37·40	13·75	23·65	16·15	60	0·50
	IS_x	84	31·28	36·0	14·6	7·59	6·0	17·45	87·05	31·40	55·65	42·1	—	2·25
							Unpricked							
1	$IIIS_1$	1	61·20	—	—	—	5·4	9·59	(28·62	15·75	10·87)	—	0	—
	$IIIS_2$	28	47·54	7·8	3·1	0·23	4·59	26·12	(44·55	23·70	20·75)	—	0	—
	$IIIS_3$	51	43·52	14·9	5·2	1·41	4·55	29·1	(40·25	26·55	13·70)	—	0	—
	$IIIS_4$	82	40·16	18·06	5·04	1·99	4·77	30·60	50·05	27·55	22·70	13·81	1	13·00
	$IIIS_5$	103	37·99	16·45	6·65	2·59	4·59	29·75	50·25	27·37	22·88	12·94	5	6·50
	$IIIS_6$	119	36·38	21·8	6·90	2·89	4·95	27·5	50·30	24·12	26·18	16·0	7	4·00
	$IIIS_7$	147	35·35	27·1	9·0	3·6	5·01	26·75	48·55	22·50	26·05	16·5	15	5·75
2	$IIIS_8$	155	31·95	29·4	9·2	4·25	4·83	29·65	51·75	21·90	29·89	14·75	25	11·00
	$IIIS_9$	167	33·5	26·9	8·5	3·9	5·10	38·25	61·05	34·30	26·75	17·87	5	14·00
	$IIIS_{10}$	183	32·97	37·1	12·4	6·64	6·23	11·00	46·20	15·90	30·30	22·44	35	15·25
	$IIIS_{11}$	195	33·09	40·8	16·4	7·89	6·07	15·25	37·75	15·30	22·45	17·37	65	18·50
3	$IIIS_{12}$	238	32·48	43·2	20·8	9·50	6·4	18·25	50·05	17·20	32·85	26·62	60	16·50
	$IIIS_x$	110	36·05	52·03	15·39	9·47	5·39	12·19	80·25	53·25	27·00	24·2	—	—

NP = non-protein; NPS = non-protein Sørensen.

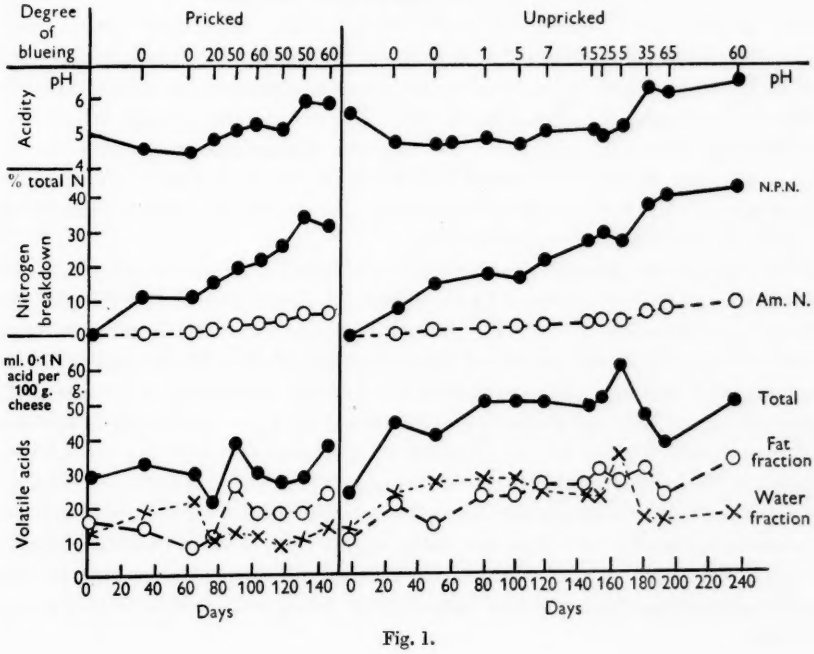

Fig. 1.

was almost at its maximum and very little difference was noted in the veining of the cut cheese from that day until the end of this series of tests on the 147th day.

Cheeses $IIIS_1$ to $IIIS_{12}$ represent another batch of Stiltons, also from one bulk of milk. No borings were taken of these cheese and, with the exception of $IIIS_x$, there was no deliberate admission of air to the interior by pricking or other means. The first signs of mould growth were found in the cheese cut at 103 days after manufacture. The mould developed slowly, but it was definitely established in the cheese examined on the 147th day after manufacture. The early mould development was much slower than in the pricked cheese. The growth in IS_5, 27 days after pricking, was more abundant than in $IIIS_{10}$, 64 days after blueing was first noted. This series was less uniform than IS, and one cheese, $IIIS_9$, showed little mould growth on 167th day after manufacture. The veining tended to be centralized and not as well distributed as is desirable.

One cheese from this batch was heavily pricked with stainless steel wire (s.w.g. 16) at 2 months after manufacture and again with a finer wire 14 days later. When this cheese, $IIIS_x$, was cut at 110 days, i.e. 52 days after the first pricking, the veins were well distributed and more abundant than in $IIIS_{12}$ at 238 days.

The ripening process of Stilton cheese may be conveniently divided into three stages: (1) the white stage (pre-mould growth), (2) the period of active mould growth, and (3) the maturation period.

(1) *The white stage*

This period is characterized by rising titratable acidity and falling pH. Although no starter, or at the most a very few drops, is used in the making of Stilton cheese and the milk is renneted while still sweet, more acidity develops in a young Stilton cheese than in a cheese of the Cheddar type. The curd is allowed to stand in its whey for several hours, a condition which is favourable to acid production. Moreover, the curd is very moist when it is placed in the hoops and, as no pressure is applied to the cheese, drainage is slow. Thus the method of manufacture of Stilton cheese is such that the curd is in close contact with a highly acid whey for a long period during manufacture and for several days of slow drainage in the hoops. As a consequence there is a considerable seepage of calcium and phosphorus from the curd into the whey, and the calcium and phosphorus content of Stilton (and other blue-veined) cheese is definitely lower than that of the hard, white varieties in which the less acid whey is quickly removed from the curd (4)—a factor which may affect the subsequent ripening process.

The volatile acidity usually varies little after the first few days, but sometimes there is a gradual rise, no doubt influenced by the types of bacteria present and the efficiency of the drainage of the whey. The acids in the water fraction exceed those in the fat fraction, and there is only a small amount of water-insoluble matter in the steam distillates. Co-incident with this acid production there is a gradual breakdown of the casein. These changes, which are indicated in the figures for IS and IIIS, are reminiscent of the changes in Cheddar cheese. Figures for examinations at shorter intervals during this white stage are shown in Table 2 for another batch of cheese, in which Cheddar (VC) and Stilton (VS) made from one bulk of milk are compared. The general trend is on the whole similar, but the moisture and acidity of Stilton are always higher than those of Cheddar cheese. This white stage may vary in length but can be definitely shortened by pricking the cheese, when the second stage follows almost immediately. In unpricked cheese it may be greatly protracted.

(2) *The period of active mould growth*

This is the period of easily recognizable changes. The blue veins make their appearance and increase in extent, and the cheese begin to acquire the characteristic smell and flavour; CO_2 produced by the metabolism of the growing mould tends to accumulate in the body of the cheese and is found in increasing quantities in the steam distillates.

Table 2

Cheese code no.		Age (days)	Moisture (%)	Protein breakdown (% total nitrogen) NP	NPS	NH_4	Acidity pH	Volatile acids, ml. N/10 acid/100 g. Direct steam dist.	Extraction method Total	H_2O fract.	Fat fract.	H_2O insol.	Degree of ripeness Blueing	CO_2 in curd
								Stilton						
VS	1	1	60·42	3·29	0·91	0·17	4·75	9·90	29·5	20·0	9·5	4·6	0	5·00
	2	3	54·44	5·79	0·76	0·15	4·49	14·38	30·0	20·0	10·0	4·38	0	3·25
	3	5	52·93	6·10	0·89	0·58	4·53	14·88	30·25	20·0	10·25	5·88	0	6·00
	4	7	63·10	4·80	1·72	0·50	4·52	16·75	28·75	19·5	9·25	4·85	0	9·25
	5	9	—	—	—	—	—	16·38	30·5	17·75	12·75	5·5	0	5·75
	6	12	—	—	—	—	—	15·75	26·5	16·25	10·25	6·0	0	8·25
	7	15	—	—	—	—	—	14·62	28·0	15·0	13·0	6·25	0	7·25
	8	18	—	—	—	—	4·78	14·00	31·25	18·75	12·25	6·38	0	6·25
	9	21	—	—	—	—	4·46	14·12	26·75	16·25	10·05	5·38	0	6·00
	10	26	—	—	—	—	4·30	15·25	28·5	15·5	13·0	6·62	0	9·75
	11	34	46·80	12·4	4·6	1·78	4·41	14·50	27·75	16·0	11·75	6·25	0	7·00
	12	42	—	—	—	—	4·28	19·0	27·25	17·25	10·0	5·5	0	16·75
	13	57	—	—	—	—	4·92	16·15	28·25	17·5	10·75	6·5	0	13·75
								Cheddar						
VC	1	1	39·84	1·86	0·65	0·16	—	6·18	22·0	12·75	9·25	4·75	—	8·50
	2	2	37·71	4·01	0·71	0·28	5·7	8·25	20·55	11·5	8·75	5·75	—	5·50
	3	4	37·27	5·79	1·12	0·50	5·11	8·0	20·5	11·25	9·25	5·10	—	4·75
	4	6	37·54	5·90	1·91	0·48	5·03	11·15	24·75	14·0	10·75	6·25	—	6·75
	5	8	37·81	5·80	2·18	1·04	4·94	12·25	23·75	14·75	8·0	5·25	—	7·25
	6	12	—	—	—	—	—	16·62	28·5	20·0	8·5	4·5	—	6·25
	7	15	—	—	—	—	—	15·15	29·5	20·0	9·5	6·0	—	7·00
	8	18	—	—	—	—	4·98	16·13	29·75	22·5	7·25	5·25	—	6·75
	9	21	—	—	—	—	4·96	17·25	35·25	23·5	11·75	6·0	—	6·25
	10	26	—	—	—	—	4·89	24·25	38·5	28·0	10·5	5·75	—	6·00
	11	34	36·18	15·3	4·5	1·35	4·94	28·75	43·25	32·25	11·0	6·0	—	6·50
	12	57	—	—	—	—	4·99	33·15	47·45	36·0	11·45	6·5	—	13·00
	13	83	36·00	22·1	9·7	2·11	5·13	38·00	50·5	37·75	12·75	7·5	—	11·50

See Table 1 legends.

Two factors, increase in the figure for CO_2 content and the degree of blueing, have been used to define this period. Unfortunately the amount of CO_2 was not estimated for the distillates of the first three cheese of the series IS and IIIS, but the data for the later analyses are useful in delimiting this period of active growth. For IS it began at the 63rd day, when the cheese were pricked. Blue mould was evident in borings taken 4 days later. The CO_2 value for IS_3 (immediately before pricking) was not estimated but it was high for IS_4 and remained so until the 104th day when the maximum value (60) for blueing was noted, after which it declined. The active period is, therefore, taken to be from the 63rd to 104th days, i.e. 41 days.

In the unpricked cheese the mould did not develop until much later and the active period, as indicated by increased CO_2 production, could be more readily defined. Traces of blue mould were found in $IIIS_5$ and $IIIS_6$ at 110 and 119 days respectively, but the blue patches were avoided when taking the samples for analysis. A distinct rise in the CO_2 content was not noted until definite blueing was observed, i.e. in the cheese $IIIS_7$ at 147 days, and the

values continued to rise until cheese $IIIS_{11}$ was examined on the 195th day, i.e. during 48 days, a period of the same order as that for the pricked cheese. These limits agree with those suggested by the degree of blueing. There was a more definite fall in the CO_2 content at the end of the period in series IS than in IIIS, probably because the pricking in the former series allowed a freer dispersal of the gases in the cheese.

There were definite chemical changes in the cheese during this period. The pH of the cheese increased, the non-protein nitrogen increased rapidly, the ammonia nitrogen definitely, but more slowly. There were changes also in the quantity and type of volatile fatty acids. A decrease in the volatile acids has repeatedly been observed when active mould growth starts in the cheese. This was apparent when direct steam distillation of the cheese was used, but the extraction method showed that there were changes in type as well as in quantity. At the time of these experiments no sure but quick method had been elaborated for the identification and estimation of the individual acids in numbers of cheeses. The extraction method was an attempt to meet this need. By separating the water-soluble from the fat-soluble types it gave an indication of the progress of lipolysis. During the white stage the acidity of the distillate from the water fraction was greater than that from the fat fraction, as has been found to be the case in all types of white cheese examined (1), but as soon as the mould began to grow the positions were reversed. The acidity of the water fraction decreased and that of the fat fraction increased to exceed that of the water fraction, and remained in excess throughout the period.

The method of removing the fat from the water emulsion of the cheese, i.e. by hand picking, was quick but crude, and it was expected that some of the acids of the water fraction would be carried over into the fat fraction. Nevertheless, since the fatty acids produced by the lipolysis of butterfat were, with the possible exception of butyric, more soluble in fat than in water, it was expected that lipolysis would be marked by an increase in the amount of the acids in the fat fraction. This was actually the case, though the increase was not usually great and was most clearly marked by the increase in the water-insoluble acids, i.e. lauric, capric, and to some extent caprylic, collected on the filter-paper placed at the mouth of the condenser. During the white stage these insoluble acids were consistently low. The recent use of paper partition chromatography (5) for the examination of distillates from the water and fat fractions of blue Stilton cheese has shown that both distillates, after removal of the insoluble acids by filtration, contained acetic, butyric, caproic and caprylic acids in varying amounts. The total amount of these acids was usually greater in the water fraction than in the fat fraction, the excess being mainly in the acetic and butyric acids. The caproic acid was low in both cases. Another volatile acid, traces of which have been detected on the chromatograms for blue-veined cheese, is valeric acid (or its isomer). Its origin and the stage at which it first appears have not yet been studied, though it appears probable that it might be derived from casein.

The mould is also a strong proteolytic agent, and during the period of active mould growth there was a progressive breakdown of the casein with the formation of peptones, amino-acids and ammonium salts, all of which, together with the volatile acids, are probably concerned in the development of the typical flavour of Stilton cheese.

It must again be emphasized that each cheese was analysed only once, and there must always be some doubt as to whether each cheese in the batch was at the same stage of development as the one under analysis—in fact it is most unlikely that there should be perfect agreement. The curves for the nitrogen breakdown and changes in acidity are so

smooth that it may perhaps be assumed that the figures denote the general trend of the ripening process. If that be so, the irregularity in the curves for the volatile acids which are obvious for both batches of cheese, may be real, and due, not so much to differences in the lipolytic activity within the cheese, as to the metabolic needs of the mould which assimilates varying quantities of the volatile acids as they are released from the cheese fat. One cheese, $IIIS_9$, appears to be an exception. In it the mould growth was much less extensive than would have been expected in comparison with other cheese of the same batch, and the fact that the water fraction contained a higher proportion of volatile acids than the fat fraction supports the view that this cheese was still in the early stages of ripening.

(3) *Period of maturation*

During the period of active mould growth the cheese remained hard in texture and bitter in flavour, with little of the characteristic flavour and body. It is probable that the true maturation process does not begin until the active growth of the mould is ending. Too few cheeses were left in either batch for a close study of this period, but the results recorded suggest that this was a more stable period with less marked changes in the factors measured in these experiments. There appeared to be a tendency for the volatile acids to accumulate. The most obvious changes were the mellowing of the texture and development of flavour. No attempt was made to isolate and identify any of the flavour constituents, but since methyl-ketones have been shown to be present in mould-ripened blue cheese (6, 7, 8), and conditions under which they are formed have been described (9), it is likely that they are formed during this stage of the ripening process when active growth is suspended and ammonium salts of the volatile acids may be expected to be present in the cheese.

The length of this maturation period, and also the length of time that can elapse before the cheese becomes overripe or even putrefied, appears to depend to a large extent on the method of manufacture. The old type of unpricked Stilton, with its wide veining, ripened slowly, but it developed a delicate and subtle flavour and was of long keeping quality. The more modern pricked type ripens more rapidly, but it often has a fine close veining, and has lost some of its subtle flavour and long keeping quality.

The two cheeses IS_x and $IIIS_x$, which were heavily pricked at 2 months after manufacture and again 14 days later, ripened abnormally, and both were overripe when analysed on the 84th and 110th day, respectively, after manufacture. At 21 days after the first pricking the figures for nitrogen breakdown for IS_x were rather higher than those for IS_8 which had been judged to be a near approach in flavour and texture to the old-type Stilton, but there was evidence of an abnormally high content of volatile acids, especially of the water-insoluble types. When $IIIS_x$ was examined at 52 days after the first pricking, there was evidence of strong proteolysis (which was in all cases greater in IIIS than in IS) and also of accumulation of volatile acids. The pH was lower (5·39) and the value for volatile acids in the water fraction was greater than that for the fat fraction, two characters which are reminiscent of Roquefort type cheese (1) rather than of Stilton.

As far as can be judged from the figures available, it would appear that by judicious pricking the ripening period of a Stilton cheese can be shortened without materially affecting the flavour and appearance of the ripened cheese. From commercial evidence it is possible that such cheese, when ripe, may have a shorter keeping quality than the unpricked, slow-ripening type. Excessive and uncontrolled pricking will almost certainly give cheese which blue quickly but ripen abnormally and are of poor keeping quality.

It may happen that for some reason the rinds of the cheese do not crack normally, nor the veins in the interior open sufficiently to provide the air conditions necessary for healthy mould growth, and such cheese may fail to blue unless pricked, even if allowed an unduly ong ripening period. The circumstances that promote these conditions in the cheese have not been established and it can only be speculated why some cheese in a batch should show these peculiar conditions and the remainder ripen quite normally. The cheese of one batch (IVS), which had been neither pricked nor bored, failed to blue even at 174 days after manufacture. It had been noted that the coats of most of these cheese were thicker than those in IIIS (also unpricked) and that there was, moreover, a thick sticky undercoat, suggestive of early incipient slip-coat which had not fully developed. The analyses of the eight cheese which had already been examined gave no indication that the curd had been unduly moist when placed in the hoops, or that the cheese had drained badly or failed to develop the correct acidity. The figures were of the same order as those of IIIS which had blued normally.

Table 3

Cheese code no.		Age (days)	Moisture (%)	Protein breakdown (% total nitrogen) NP	NPS	HN_4	Acidity pH	Volatile acids ml., N/10 acid/100 g. Direct steam dist.	Extraction method Total	H_2O fract.	Fat fract.	H_2O insol.	Degree of ripeness Blue-ing	CO_2 in curd
								Unpricked						
IWS	1	1	61·28	—	—	—	5·46	8·22	(25·95	14·2	11·75)	—	0	—
	2	37	44·78	8·5	3·0	0·41	4·46	18·80	(38·7	22·05	16·75)	—	0	—
	3	63	41·09	13·50	3·22	1·39	4·76	20·40	35·75	20·30	15·45	9·05	0	12·0
	4	91	39·11	14·58	5·45	1·57	5·00	22·64	37·87	24·37	13·50	8·50	0	1·20
	5	121	37·02	16·0	5·4	2·31	4·85	20·02	45·00	23·70	19·42	9·50	0	—
	6	146	34·82	19·4	6·3	2·51	4·81	26·5	50·70	27·75	22·45	9·70	0	11·5
	7	160	33·26	18·00	7·3	3·55	5·05	29·37	45·50	31·12	14·38	9·25	0	14·5
	8	174	32·07	17·1	7·8	2·47	5·13	30·75	48·60	28·25	20·35	9·87	0	15·00
W	9	287	26·97	21·9	9·5	3·85	—	33·10	55·25	31·19	24·06	12·25	1	3·2
B	10	289	27·81	31·8	12·1	4·83	—	25·00	46·56	24·19	22·37	16·9	15	5·00
SS	11	286	27·71	30·3	12·3	5·77	—	24·25	51·62	21·62	30·00	19·05	20	4·00
P	12	286	28·07	30·3	12·8	4·53	—	24·5	54·65	23·08	31·58	20·4	30	3·5

W = control (white); *B* = control (blueing); *SS* = ringed, *P* = pricked.

It is probable that this sticky undercoat acted as a barrier to the free exchange of gases between the atmosphere and the interior of the cheese. The concentrations of CO_2 and O_2 in the interior of the cheese are determining factors in mould growth (10), and it may be that the failure of excess CO_2 to escape from, and of O_2 to penetrate to, the interior of the cheese, had played an important part in preventing normal mould growth. Four cheese of this batch still remained and the undercoats of two of them were pierced, one by ringing with a sharp knife, removing the coat and undercoat to a width of about $\frac{1}{4}$ in., the other by lightly pricking with a stainless steel needle (s.w.g. 16). After a further ripening period of about 110 days, the ringed cheese was blueing centrally, the pricked cheese in well-distributed veins. One of the controls showed occasional traces of mould, the other had remained white.

The figures for the volatile acids of these four cheese show gradations which are typical of the series of changes from the white stage to full development of the mould, i.e. the decline in the water-soluble and the increase in the fat-soluble acids characteristic of progressive lipolysis in this type of cheese.

There is little published work dealing with the series of bacterial and chemical changes which lead to the development of the characteristic coat of Stilton cheese, but it is known

that there is successive growth of bacteria, yeasts and moulds of the *Oidium* and *Candidum* type on the surface. It is generally accepted that these yeasts and moulds are chiefly responsible for the dry crinkled coat, but few data are available to establish this or to indicate the reasons for the difference between this and the moist, sticky, reddish coat found on many modern Stiltons. It is possible that the latter type of coat does not dry and crack as does the crinkled coat, and that pricking is necessary to ensure satisfactory mould development in such cheese. Moreover, it is possible that the organisms growing on the surface may, as in the various 'smear' cheeses, more directly influence the course of the ripening process. Recent researches by Morris, Combs & Coulter(11) suggest that the development of the slimy coat of Minnesota Blue cheese (Roquefort type) directly or indirectly influence the volatile acidity, pH and nitrogen breakdown of the interior of the cheese.

The underlying cause of 'slip-coat' is equally uncertain. It is popularly associated with slow drainage of the young cheese and the development of conditions which favour the abnormal growth of proteolytic organisms in the outer layers. In extreme cases the casein is liquefied to such a degree that the coat slides away from the body of the cheese. It

Table 4

Cheese		Calcium content (%) Crumb	Coat	Cheese		Calcium content (%) Crumb	Coat
I N	1	0·31	1·0	I L	1	0·23	1·1
	2	0·33	1·2		2	0·22	1·1
	3	0·29	1·1		3	0·26	1·4
	4	0·30	1·2		4	0·28	1·3
II N	5	0·13	0·79	II L	5	0·10	0·62
	6	0·14	0·63		6	0·17	0·45
	7	0·12	0·75		7	0·11	0·99
	8	0·15	0·81		8	0·11	0·44
III N	9	0·10	0·40	III L	9	0·14	0·35
	10	0·19	0·30		10	0·14	0·29
	11	0·11	0·46		11	0·10	0·35
	12	0·12	0·38		12	0·07	0·54
IV N	13	0·16	0·93	IV L	13	0·13	0·31
	14	0·15	0·57		14	0·12	0·37
	15	0·17	0·71		15	0·13	0·69
	16	0·15	0·57		16	0·15	0·65

would appear, however, that in some cases these undesirable changes are checked before this stage is reached, and the cheese seem to recover and form normal coats. It is possible that in such cases this semi-digested layer persists as a thick sticky undercoat, such as is sometimes found in outwardly normal cheese (cf. group IVS), but experimental evidence for this suggestion is lacking.

It is not known to what extent the distribution of mineral salts within a Stilton cheese may affect the flora of the interior and the coat. Hitherto unpublished records of the late Dr E. C. V. Mattick(12) show that there is a considerable concentration of calcium and phosphorus in the whey which drains from a young Stilton, and irregular drainage may lead to undue concentration of these and other minerals in various parts of the cheese. However that may be, there is undoubtedly an accumulation of calcium in the coats of ripe Stiltons. This is indicated in the analytical data for the rinds of three Stilton cheese given by Davies(13), and confirmed by the later intensive researches of Mattick. For the purpose of this paper some of her figures have been recalculated to show the relative concentrations of calcium in the crumb and the coat. That for the crumb varied from

0·10 to 0·33%, for the coat from 0·31 to 1·4%. The concentration in the coat was from three to six times that in the crumb and, of the small quantity of residual calcium in the cheese, from 40 to 70% was situated in the coat. The figures quoted by Mattick do not refer to the cheese (IS, IIIS, IVS, VS) dealt with in this paper, but the distribution of calcium is no doubt of the same order in all Stilton cheese manufactured by the long-established technique.

SUMMARY

The changes in the volatile acidity and other factors during the ripening process of Stilton cheese have been studied. The growth of the mould was characterized by an increase in the fat-soluble fatty acids derived from the cheese fat.

Pricking the cheese ensured a more uniform blueing and hastened the ripening. There was no evidence that judicious pricking at 2 months after manufacture seriously affected the course of the ripening. Excessive pricking, however, produced abnormal cheese of poor keeping quality.

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456. THE CALCIUM AND PHOSPHORUS OF CHEDDAR AND STILTON CHEESE

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(With 4 Figures)

I. CHEDDAR AND STILTON CHEESE OF NORMAL MANUFACTURE

In an earlier publication (1) it was shown that the proportion of the original calcium and phosphorus of the milk retained in ripe cheese of different types varied considerably, the loss of calcium during manufacture of unpressed cheese being very high. As experiments, designed to test the possibility of introducing some change in manufacturing methods which might result in reducing this loss, were in progress, it was necessary to determine at which stage this loss was greatest. In an attempt to do this two batches of cheese, one hard pressed—Cheddar—and one unpressed—Stilton—were made from the same bulk milk. Three Cheddars and nine Stiltons were made from the respective curds.

In the previous experiment (1), samples of whey were taken: (1) at the cutting (or ladling) of the curd, (2) at the drawing of the whey, (3) after hooping or putting away, with one additional overnight draining of whey from the Stilton before hooping. Samples of curd were taken at: (1) cutting (or ladling), (2) at drawing of whey, (3) at putting away, (4) at 3 days (and 65 days for Stilton), (5) at 4 months (and 6 months for Stilton), and (6) at 8 months.

Table 1

	Milk		Starter	
	Liquid	Dry matter	Liquid	Dry matter
Titratable acidity	0·175	—	0·90	—
Total solids	12·10	—	13·16	—
Ash (%)	0·765	6·32	0·78	5·93
Calcium (%)	0·120	0·99	0·131	1·00
Phosphorus (%)	0·104	0·86	0·115	0·87

Table 1A

Type of cheese	Wt. of milk (lb.)	Wt. of starter (lb.)	Wt. of Ca (lb.)	Wt. of P (lb.)
Cheddar	600	6	0·81	0·68
Stilton	530	(10 ml.)	0·64	0·55

In the present experiment the titratable acidity of the whey was determined frequently, and whenever a definite change in this value was observed for the Stilton whey, a sample was taken for estimation of calcium. It is unfortunate that the practical difficulties made the collection and weighing of bulk whey impossible. Only the percentage composition of samples can therefore be given, but determination of the moisture content allows a calculation of the percentage of calcium and phosphorus in the dry matter at each stage.

The composition of the milk and starter is shown in Tables 1 and 1A, from which it is seen that milk and starter for the Cheddar cheese contained 0·81 lb. of calcium and 0·68 lb.

of phosphorus before renneting, while that for the Stilton contained 0·64 lb. of calcium and 0·55 lb. of phosphorus. The amounts of calcium and phosphorus in the starter used with the Stilton milk was so small that its weight may be neglected.

Tables 2 and 3 (Figs. 1 and 2) show that there was a small but definite increase of calcium and phosphorus in the whey from the Cheddar cheese during the 2 hr. between cutting of curd and drawing of whey, which was reflected in slightly lower values in the curd, while the titratable acidity also increased from 0·14 to 0·26%.

There were no whey drainings from the cheese 2 hr. after drawing off the main bulk, and the next sample was taken after the cheese had been in the press for 16½ hr. The

Table 2. *Cheddar cheese—whey*

Stage of manufacture	Time	Titratable acidity N/9-NaOH	Total solids (%)	Liquid whey (%)			Dry matter (%)			
				Ash	Ca	P	Ash	Ca	P	Ca/P
Milk (+starter) before renneting	10.25 a.m.	0·20	11·9	0·76	0·122	0·102	6·42	1·02	0·88	1·17
Whey at cutting curd	10.55 a.m.	0·14	6·97	0·58	0·047	0·052	8·26	0·67	0·74	0·91
Whey at pitching curd	12.20 p.m.	0·18	6·82	0·60	0·050	0·050	8·81	0·74	0·73	1·00
Whey at drawing	12.55 p.m.	0·26	6·84	0·60	0·053	0·055	8·83	0·78	0·81	0·96
Whey from press	Next day, 9.30 a.m.	0·825	16·07	8·66	0·283	—	53·89	1·76	—	—

Table 3. *Cheddar cheese—curd*

Stage of manufacture	Time	Total solids (%)	Moist curd (%)			Dry matter (%)			
			Ash	Ca	P	Ash	Ca	P	Ca/P
At cutting	10.55 a.m.	18·76	1·00	0·219	0·164	5·36	1·17	0·88	1·34
At pitching	12.20 p.m.	42·25	1·65	0·489	—	3·92	1·16	—	—
At drawing of whey	12.55 p.m.	55·05	1·97	0·595	0·425	3·57	1·08	0·77	1·40
At second turning	2.30 p.m.	57·77	2·19	0·656	—	3·80	1·13	—	—
At third turning	3.30 p.m.	57·75	2·19	0·665	—	3·78	1·15	—	—
At milling	3.45 p.m.	57·55	2·18	0·657	—	3·80	1·14	—	—
Ripe cheese	131 days	65·81	3·60	0·683	—	5·47	1·01	0·76	1·32

acidity and percentage of ash and of calcium in this sample were very high in comparison with those taken at the drawing of bulk whey, but as the volume was small the loss was not great, as can be seen from Tables 3 and 6. The high ash was, of course, due to the salt added at time of hooping.

The results for Stilton cheese were entirely different (Tables 4 and 5, Figs. 3 and 4). At the time the curd was ladled, the percentages of calcium and phosphorus in the dry matter of the whey were 0·43 and 0·49 respectively. Two hours later these had increased to 0·69 and 0·76, after another 2¼ hr. the values were 0·98 and 0·88, and by the time the curd was turned out of the bundles, 7½ hr. after ladling, the percentage of calcium and phosphorus in the dry matter of the whey had increased to 1·8 and 1·1 respectively, while the calcium/phosphorus ratio had increased from 0·89 to 1·70, indicating a relatively greater removal of calcium than of phosphorus from the curd. This was associated with a further increase in titratable acidity from 0·115 to 0·485%. A further increase in calcium and phosphorus in the whey, an increase in titratable acidity to 0·965 and a rise in the calcium/phosphorus ratio to 2·11 took place during the next 16 hr., until the curd was broken ready for hooping. The whey drainage from the moulds after the first 8 hr. showed a great increase in ash from 14·6 to 41·5% which was largely due to the salt added at the time of salting, as are the

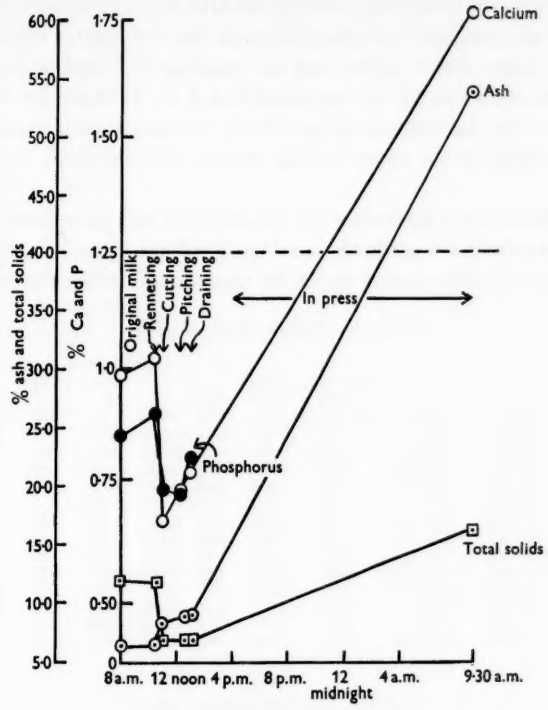

Fig. 1. Analysis of whey—Cheddar.

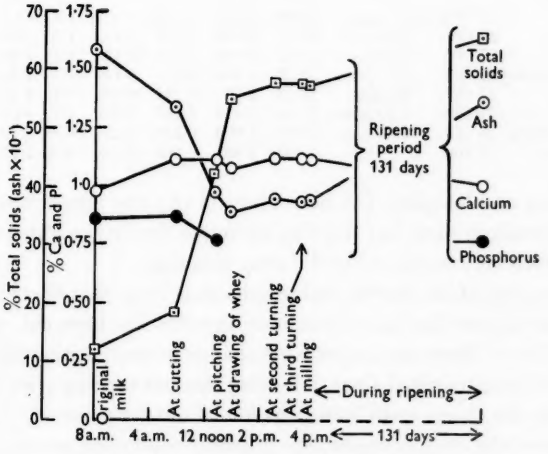

Fig. 2. Analysis of curd—Cheddar.

slightly lower percentage values for calcium and phosphorus. Slight variations occurred in the drainings during the next 5 days, but on the fifth day, 21½ hr. after the first scraping, the percentage of ash, calcium and phosphorus in the dry matter differed only slightly from those of the whey which seeped out on breaking the curd at hooping, while the calcium/phosphorus ratio was 2·10 as compared with 2·11. It would be of interest to know whether the rise in the calcium/phosphorus ratio represents further degradation of the casein fraction or what is the source of the excess calcium which is leached out with rising acidity.

From Table 5, which gives figures for the ash, calcium and phosphorus in the curd, it is seen that the ash was reduced gradually until the curd was turned out of its bundles, but that there was a big reduction during the 16 hr. that elapsed before the curd was broken in

Table 4. *Stilton cheese—whey*

					Liquid whey				Dry matter			
Stage of manufacture	Date	Time	Titratable acidity N/9-NaOH	Total solids (%)	Ash (%)	Ca (%)	P (%)	Ca/P	Ash (%)	Ca (%)	P (%)	Ca/P
Ladling of curd	27 Oct.	10.2 a.m.	0·115	10·2	0·58	0·044	0·050	0·88	5·66	0·433	0·486	0·89
About 2 hr. later	27 Oct.	11.55 a.m.	0·130	6·63	0·59	0·046	0·050	0·92	8·84	0·693	0·760	0·91
At draining	27 Oct.	2.30 p.m.	0·195	6·60	0·62	0·059	0·058	1·02	9·38	0·898	0·880	1·02
About half an hour later	27 Oct.	2.55 p.m.	0·33	6·66	0·69	0·087	0·069	1·26	10·32	1·306	1·037	1·26
About an hour later	27 Oct.	4.0 p.m.	0·38	6·76	0·79	0·123	0·082	1·50	11·61	1·812	1·209	1·50
At turning out	27 Oct.	5.30 p.m.	0·485	7·58	0·82	0·137	0·081	1·69	10·82	1·813	1·068	1·70
Drained overnight	28 Oct.	9.0 a.m.	0·710	7·02	0·95	0·184	0·101	1·82	13·52	2·620	1·440	1·82
At breaking curd	28 Oct.	9.30 a.m.	0·965	8·06	1·18	0·275	0·130	2·12	14·63	3·407	1·615	2·11
Draining from moulds	28 Oct.	4.50 p.m.	0·93	10·42	4·32	0·268	0·151	1·77	41·46	2·568	1·446	1·78
Whey from moulds	29 Oct.	10.00 a.m.	1·10	10·44	4·07	0·299	0·167	1·79	39·02	2·859	1·603	1·78
Whey from moulds	30 Oct.	10.30 a.m.	1·25	11·07	4·25	0·353	0·178	1·98	38·42	3·184	1·612	1·97
Whey from moulds	1 Nov	9.30 a.m.	2·00	17·99	7·09	0·564	0·264	2·14	39·39	3·132	1·464	2·14
After scraping and returning to moulds at 3 p.m. on 2 Nov.	3 Nov.	10.30 a.m.	1·50	13·63	5·66	0·442	0·210	2·10	41·55	3·239	1·542	2·16

Table 5. *Stilton cheese—curd*

				Moist curd				Dry matter			
Stage of manufacture	Date	Time	Total solids (%)	Ash (%)	Ca (%)	P (%)	Ca/P	Ash (%)	Ca (%)	P (%)	Ca/P
At ladling	27 Oct.	10.2 a.m.	12·60	0·804	(0·135)	(0·109)	1·2	6·38	1·070	0·866	1·2
About 2 hr. later	27 Oct.	11.55 a.m.	16·58	0·936	0·184	0·149	1·2	5·64	1·108	0·898	1·2
At drawing whey	27 Oct.	12.30 p.m.	19·72	0·946	0·192	0·135	1·4	4·80	0·972	0·685	1·4
About half an hour later	27 Oct.	2.55 p.m.	25·23	1·108	0·259	0·197	1·3	4·39	1·026	0·782	1·3
About an hour later	27 Oct.	4.0 p.m.	26·53	1·103	0·261	0·205	1·3	4·16	0·984	0·772	1·3
At turning out	27 Oct.	5.30 p.m.	21·50	0·879	0·196	0·169	1·2	4·09	0·913	0·785	1·2
At breaking (before salting)	28 Oct.	9.30 a.m.	40·35	1·003	0·228	0·257	0·89	2·49	0·564	0·636	0·89
Ripe cheese (131 days)	7 Mar.	—	71·93	1·807	0·066	0·261	0·25	2·51	0·091	0·363	0·25

readiness for salting and hooping. The percentage in the ripe cheese was almost identical with that of the (unsalted) curd; but this was, of course, due to the added salt since calcium and phosphorus were very much reduced during this time.

From a consideration of the figures and graphs, it is clear that there is little change in the calcium content of the Cheddar cheese, once the curd has been cut, until salt is added after milling; neither is there any appreciable change in composition during the ripening period. This was to be anticipated since, following the first turning after pitching, no more whey drained from the cheese until some was forced out by pressing.

But, in the case of the Stilton cheese, the different conditions are clearly evidenced by the different results. From the time when the curd is ladled there is a constantly increasing percentage loss of minerals, particularly calcium, in the whey, until it ceases to drain from the moulds. In this cheese, samples were taken over an 8-day period; each analysis showed

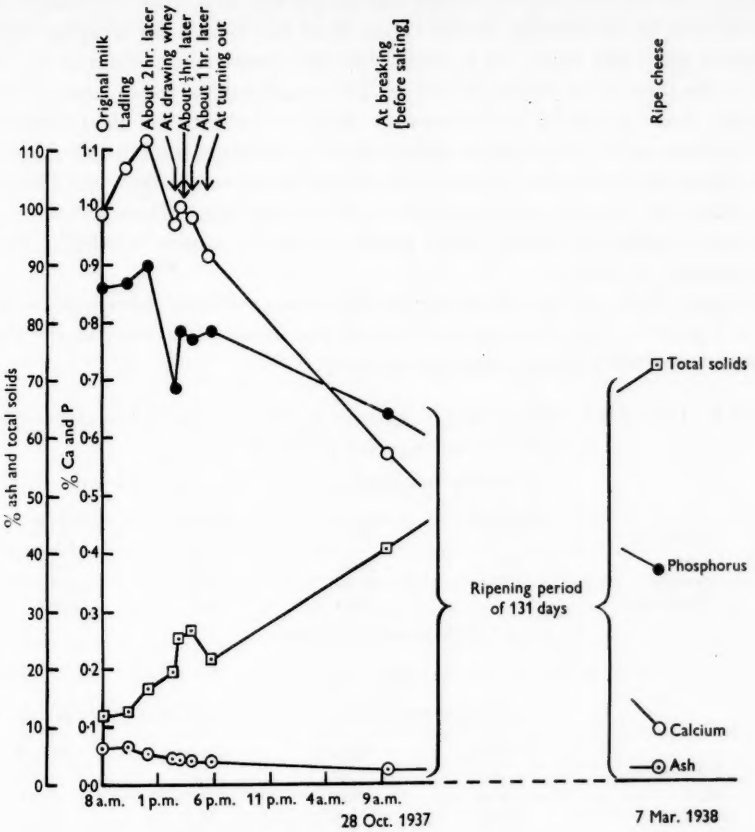

Fig. 3. Stilton cheese—curd.

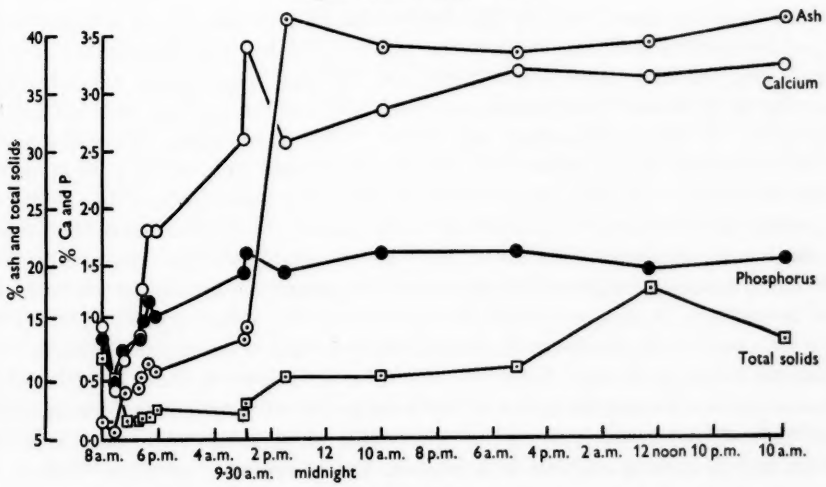
% ash and total solids
% Ca and P
Ash
Calcium
Phosphorus
Total solids
8 a.m. 6 p.m. 4 a.m. 2 p.m. 12 10 a.m. 8 p.m. 6 a.m. 4 p.m. 2 a.m. 12 noon 10 p.m. 10 a.m.
9·30 a.m. midnight

Fig. 4. Stilton cheese—whey.

an increase over the previous one except that on the last day, when the conditions were slightly altered by the removal of the cheese from the moulds for scraping before the collection of whey was made. It is found that this increase in calcium in the whey is parallel to the increase of titratable acidity. The actual mineral losses during the manufacture and ripening of these two cheeses are shown in Table 6. The final values are not strictly accurate, as the weight of the individual daily drainings from the moulds and from the ripe cheese are not known, but since the values for the total solids were known, comparable values for calcium and phosphorus could be calculated. These values are, moreover, known to indicate a slightly higher percentage for the mineral salt left in the cheese than is actually the case.

These figures show considerable agreement with those previously given (1), some of which, shown in Table 6A, have been converted from percentages remaining in the cheese to percentages lost during manufacture and ripening.

Table 6. *Loss of ash, calcium and phosphorus in Cheddar and Stilton cheese during manufacture and ripening period of* 132 *days*

	At 'milling' and 'breaking'		After 132 days	
Substance	Cheddar (%)	Stilton (%)	Cheddar (%)	Stilton (%)
Ash*	67·3	74·81	62·46	77·51
Calcium	39·5	63·68	42·5	94·10
Phosphorus	—	52·63	50·7	72·96

* Includes salt in ripe cheese.

Table 6A

	At milling and breaking		After 4 months' ripening	
Substance	Cheddar (%)	Stilton (%)	Cheddar (%)	Stilton (%)
Ash	68·49	75·07	60·55	80·64
Calcium	39·22	67·83	43·14	92·70
Phosphorus	37·42	56·04	49·69	73·63

Tables 6 and 6A show definitely that by the time the Cheddar cheese is milled nearly 40% of the calcium and phosphorus in the milk used in their manufacture has been lost in the whey, but little more is lost during the ripening, since, during the subsequent 4 months these figures only increase to about 43% of the calcium and 50% of the phosphorus. At the corresponding stage in the Stilton manufacture, when the curd is broken, salted and hooped, about 65% of the calcium and 55% of the phosphorus has already been removed in the whey and 4 months later only about 6–7% of the calcium and 17% of the phosphorus of the milk are left in the cheese. A consideration of the relationship between the titratable acidity of the whey and the solubility of the mineral salts points to the fact that there is no particular stage in normal present-day methods at which this loss could be avoided. If, however, a satisfactory cheese of the Stilton type could be made in which little acidity was allowed to develop in the whey, more of the original milk salts might be retained in the ripe cheese. Another source of loss is discussed in Part II of this paper.

Various cheese. During the course of this work an opportunity occurred for analysis of some kinds of cheese which are not always readily obtainable. A comparison of their calcium and phosphorus contents is of interest. The percentages, calculated both on the cheese as eaten, and on the dry matter, are shown in Table 7.

Table 7. *Percentage of calcium and phosphorus in various types of cheese when ripe*

Type of cheese	Cheese as eaten				Dry matter in cheese			
	Ash (%)	Ca (%)	P (%)	Ca/P	Ash (%)	Ca (%)	P (%)	Ca/P
P, Sheep's Milk—Portuguese	—	0·634	0·471	1·35	—	1·146	0·880	1·30
P_1, Sheep's Milk—Portuguese	—	0·713	0·501	1·42	—	1·279	0·898	1·42
P_2, Sheep's Milk—Portuguese	—	0·675	0·484	1·40	—	1·194	0·856	1·40
Cheddar—N.Z. tinned	—	0·629	0·461	1·36	—	0·925	0·678	1·36
R_1, French Roquefort	—	0·411	0·401	1·03	—	0·677	0·650	1·04
Cheshire—Blue	3·98	0·562	0·449	1·25	6·55	0·785	0·627	1·25
G_1, Gorgonzola	—	0·330	0·318	1·04	—	0·598	0·577	1·04
D_2, Rokanzola	5·22	0·387	0·361	1·07	8·64	0·643	0·598	1·08
O, Goat milk—Norwegian	—	0·326	0·558	0·58	—	0·480	0·821	0·58
IS 146,* Stilton Blue Pricked	2·49	0·326	0·336	0·97	3·62	0·475	0·489	0·97
SH, Stilton	—	0·166	0·266	0·62	—	—	—	—

* This cheese was only 3 months old and would not normally have been considered ready for market, but it was well blued and of fairly good texture. The flavour, however, was very bitter except in the depths of the mould. It had been heavily pricked and blued very rapidly, and the volatile acid content was more reminiscent of that found in a Roquefort than in an ordinary unpricked Stilton cheese.

SUMMARY

The loss of calcium and phosphorus during the manufacture of hard pressed (Cheddar) and unpressed (Stilton) cheese made from the same bulk of milk has been studied. It is found that there is little loss of mineral salts from the hard pressed variety either after cutting the curd during making, or while the cheese is ripening. In the case of the unpressed variety there is constantly increasing percentage loss of minerals, correlated with an increase of titratable acidity in the whey, until the last stages of manufacture when the cheese are removed from the hoops for scraping.

The figures confirm those previously found (1) that in the cheese examined 57–58% of the calcium and 50–51% of the phosphorus present in the milk originally used, remains in the hard pressed ripe cheese, but that only 6–8% of the calcium and 27% of the phosphorus are left in the unpressed variety when ripe.

II. THE DISTRIBUTION OF CALCIUM BETWEEN THE CRUMB AND COAT OF STILTON CHEESE

Slight differences in the methods of making Stilton cheese are occasionally introduced, and amongst these are variations in the length of time during which the curd is allowed to remain in the whey, a longer bathing supposedly producing a better cheese. During the autumn of 1939, forty-eight Stilton cheese in six groups of eight were made in the Institute dairy, in one half of which the curd was kept in the whey for a normal time, while in the other half the curd was allowed to remain in the whey for an abnormally long time. As the 'leaching out' of the calcium salts during the manufacture of Stilton cheese is obviously due to their solution in the increasingly acid whey, it was thought desirable to make analyses of these cheese. The findings form the subject of this paper.

In each experiment two sets of four cheese were made from the same bulk milk, and the usual methods of making were used until the time of ladling the curd, which was then divided as accurately as possible by alternate ladling into two different lots. One lot was bundled and drained in the usual way (normal), while the second lot of bundles were allowed to remain in the whey overnight (long time in whey = L.T.W.). After the whey was

drained off, this curd was cut at the same time as, and treated similarly to, the normal curd until the completion of manufacture. As nearly as possible the curds were hooped at the same acidities, and it was interesting to note that after the drawing of the whey acidity developed in both sets of cheese at practically the same rate. Attempts at weighing all the whey were not very successful, and the calcium and phosphorus figures at hooping are therefore based on the percentage found in the curd. Some estimations were made on groups 1, 2 and 4 during making, and the results are given in Table 8. The total amounts of calcium and phosphorus which have already been lost in the whey from these cheese during manufacture until the time of hooping is given in Table 9. A calculation of the amount lost during draining for 10–14 days after hooping was also made. The weight of

Table 8

Batch no.	Wt. of milk (lb.)	Ca in milk (lb.)	P in milk (lb.)	Wt. of curd (lb.)		Time in whey (hr.)		Ca in curd (lb.)		P in curd (lb.)		Acidity of whey at hooping: Titratable ml. N/9-NaOH		pH	
				Normal	L.T.W.	Normal	L.T.W.	Normal	L.T.W.	Normal	L.T.W.	Normal	L.T.W.	Normal	L.T.W.
1	1089·6	1·32	1·10	101·4	101·6	5	21	0·41	0·39	0·30	0·29	3·9	4·3	6·1	5·9
2	1003·9	1·26	1·02	96·4	103·4	1¼	17½	0·43	0·28	0·33	0·24	6·73	6·85	5·5	5·2
4	981·5	1·15	1·00	107·6	107·6	3	21	0·35	0·30	0·27	0·28	4·52	4·68	5·9	5·75

Table 9. *Amount of calcium and phosphorus lost during manufacture until the time of hooping*

Batch no.	Ca lost in whey (lb.)	Ca lost in whey (%)	P lost in whey (lb.)	P lost in whey (%)
1	0·80	39·4	0·59	46·4
2	0·71	43·65	0·57	44·1
4	0·65	43·5	0·55	52·2

Table 10. *Amount of calcium and phosphorus lost during draining period after hooping*

Batch no.	Normal or L.T.W.	No. of days' collection	Wt. of Ca in curd (lb.)	Wt. of P in curd (lb.)	Wt. of Ca in drainings (lb.)	% of Ca lost in drainings	Wt. of P in drainings (lb.)	% of P lost in drainings	Wt. of whey (lb.)
2	Normal	10	0·43	0·32	0·105	24·4	0·062	19·4	26·0
	L.T.W.	10	0·27	0·23	0·094	34·8	0·054	23·5	26·1
4	Normal	4	0·31	0·24	0·030	9·7	0·020	8·3	13·78
	L.T.W.	4	0·27	0·25	0·027	10·0	0·020	8·0	11·65
	Normal	9	0·28	0·22	0·054	19·3	0·038	17·3	12·87
	L.T.W.	(Subsequent to 4)	0·24	0·23	0·063	26·3	0·050	21·7	15·89
4	Normal	13	0·59	0·46	0·084	27·1	0·058	24·2	26·65
	L.T.W.	(4 + 9 as above)	0·41	0·48	0·090	33·3	0·070	25·9	27·54

curd in each batch at hooping was 96 lb. and the estimations of calcium and phosphorus were made on the whey. It was very difficult to get satisfactory samples for analysis from these first drainings because of the amount of solid and fat which was always present in the whey at this stage. Sometimes it was necessary to pour the whey through muslin before a sample could be pipetted.

Batch 5 drained badly and had to be pricked on the tenth day. In every case, except that of phosphorus after 4 days, the percentage of both calcium and phosphorus lost is greater in the L.T.W. than in the normal cheese.

The first batch to be selected for examination was no. 5. These were only 3½ months old, were quite unripe and showed no mould formation. No analysis of this group had been made during manufacture, but at 3½ months the coats appeared unsatisfactory, having something of a 'slip coat' appearance, and therefore analysis of crumb and coat ground

together, of crumb only and—in one case—of coat only were made, and gave a somewhat surprising result. The figures can be expressed only as percentage values of the cheese at time of sampling as, at this stage, separate weighings of coat and crumb were not made. The figures are shown in Table 11.

These figures indicate two facts. First, that nearly all the values in the normal cheese were higher than those in the corresponding 'L.T.W.' batch, noticeably so in calcium and less noticeably but still definitely in phosphorus, and secondly that the percentage of calcium in the coat of normal cheese no. 20 was much higher than that in the crumb. As

Table 11. *Analysis of batch* 5—3½ *months old*

	Calcium (%)					Phosphorus (%)			
	Normal			Long time in whey		Normal		Long time in whey	
Cheese no.	Crumb and coat together	Crumb only	Coat only	Crumb and coat together	Crumb only	Crumb and coat together	Crumb only	Crumb and coat together	Crumb only
XVII	0·429	—	—	0·310	0·129	0·355	—	0·321	0·236
XVIII	0·353	0·168	—	0·276	—	0·316	0·241	0·312	—
XIX	0·403	—	—	0·310	0·150	0·339	—	0·325	0·238
XX	0·354	0·136	0·692	0·286	—	0·329	0·254	0·304	—

Table 12. *Calcium in crumb and coat of cheese in batches* 3 *and* 6

Batch no.	Time in whey (hr.)	Acidity at hooping N/9-NaOH	Wt. of cheese (lb.)	Wt. of crumb (lb.)	Wt. of Ca in crumb (lb.)	Wt. of coat (lb.)	Wt. of Ca in coat (lb.)	Total wt. of Ca in cheese (lb.)	% of Ca in crumb (lb.)	% of Ca in coat (lb.)	% of moisture at hooping
					Normal time in whey						
3	3	8·6	10·49	7·21	0·0076	3·28	0·0134	—	—	—	64·7
			10·07	7·66	0·0140	2·41	0·0074	—	—	—	—
			10·89	7·61	0·0084	3·28	0·0152	—	—	—	—
			10·28	7·59	0·0090	2·69	0·0102	0·0852	45·8	54·2	—
					Long time in whey						
	21	8·35	10·35	7·31	0·0105	3·04	0·0104	—	—	—	63·5
			9·96	6·82	0·0093	3·14	0·0091	—	—	—	—
			10·12	7·21	0·0074	2·91	0·0102	—	—	—	—
			10·31	7·76	0·0053	2·55	0·0139	0·0761	42·7	57·3	—
					Normal time in whey						
6	3	10·0	11·82	8·62	0·0114	3·20	0·0137	—	—	—	60·7
			12·12	9·09	0·0138	3·03	0·0196	—	—	—	—
			10·99	8·42	0·0143	2·57	0·0135	—	—	—	—
			11·28	8·46	0·0167	2·82	0·0181	0·1211	46·4	53·6	—
					Long time in whey						
	21	10·0	10·89	8·45	0·0183	2·44	0·0157	-	—	—	61·25
			10·58	8·55	0·0126	2·03	0·0097	—	—	—	—
			10·63	8·53	0·0151	2·10	0·0107	—	—	—	—
			10·97	7·91	0·0188	3·06	0·0098	0·1107	58·5	41·5	—

only small samples were available it was decided to investigate this point more particularly for both types of cheese.

In batches 3 and 4 no analyses had been made during manufacture, but the relative weights of coat and crumb were recorded and calcium was estimated separately in each sample. Results are shown in Table 12. The cheese in batch 3 were 8 months, and those in batch 6 were 6 months old at the time the analyses were made.

In taking samples of coats both the outer- and the under-coat were removed, so that nothing remained but the edible part of the cheese. The depth (and consequent relative weight) of the coats is of course influenced by the amount of drainage before hooping

which is dependent upon the time between ladling and hooping and on the development of acidity. It is interesting to note that in only one set does the distribution of the calcium between crumb and coat show a smaller percentage weight of the total in the coat than in the crumb, viz. batch 6 L.T.W., and it is recorded of these cheese that those which remained a longer time in the whey showed, when ripe, dried outer and much thinner under-coats than those of the normal group, which almost certainly accounts for the distribution of the calcium, the weight of coats being only 28·8% of crumb. In the other three groups shown in this table, it is seen that, of the total weight of calcium in the cheese, more than 50% is found in the coat, although these represent only 38·8, 39·9, 33·6% by weight of the crumb.

Table 13. *Differences in calcium content of 'Normal' and 'Long time in whey' ripe cheese, and amounts of calcium lost during drainage in hoops and a 6 months' ripening period. Weight of curd in each hoop was 24 lb.*

Batch no. and cheese no.	Acidity at hooping N/9-NaOH	% of moisture in curd at hooping (lb.)	Ca in curd at hooping (lb.)	Wt. of ripe cheese (lb.)	Wt. of crumb (lb.)	Wt. of Ca in crumb (lb.)	Wt. of coat (lb.)	Wt. of Ca in coat (lb.)	Total weight of Ca (lb.)	% of total in crumb	% of total in coat	% lost during draining and ripening	% lost by removal of coats and in manufacture
1 *I* N	3·9	66·5	0·39	*10·11	7·91	0·0248	2·20	0·0221					
II				10·00	7·74	0·0255	2·26	0·0270					
III				10·04	7·76	0·0228	2·28	0·0258					
IV				10·05	8·04	0·0240	2·01	0·0242	0·1962	49·5	50·5	49·7	75·1
1 *I* L.T.W.	4·3	67·1	0·37	9·93	7·06	0·0162	2·87	0·0305					
II				9·82	7·78	0·0171	2·08	0·0227					
III				10·00	7·93	0·0208	2·07	0·0294					
IV				9·55	7·64	0·0216	1·91	0·0250	0·1833	41·30	58·70	50·5	79·5
2 *V* N	6·73	60·95	0·43	10·80	8·20	0·0107	2·63	0·0204					
VI				11·14	8·05	0·0114	3·09	0·0189					
VII				10·67	8·22	0·0104	2·45	0·0179					
VIII				11·32	8·33	0·0122	2·99	0·0244	0·1263	35·4	64·6	70·6	89·6
2 *V* L.T.W.	6·85	59·75	0·27	10·89	8·10	0·00842	2·79	0·0175					
VI				10·62	8·08	0·00865	2·54	0·0112					
VII				11·49	8·40	0·00890	3·09	0·0308					
VIII				11·42	8·67	0·00923	2·75	0·0118	0·1065	33·05	66·95	60·6	87·0
4 *XIII* N	4·52	64·7	0·31	10·22	8·01	0·0129	2·21	0·0205					
XIV				9·94	7·03	0·0105	2·91	0·0172					
XV				10·10	7·68	0·0132	2·42	0·0171					
XVI				10·12	7·79	0·0114	2·33	0·0131	0·1159	41·42	58·58	62·6	84·5
4 *XIII* L.T.W.	4·68	63·5	0·27	9·51	7·34	0·00947	2·17	0·00692					
XIV				8·61	6·70	0·00791	1·91	0·00701					
XV				8·82	7·14	0·00893	1·68	0·01172					
XVI				8·36	6·54	0·01007	1·82	0·01178	0·07381	58·73	41·27	72·7	85·2

* The weight of sample and of coat were, unfortunately, not recorded for this cheese, but as the group was so uniform a calculated value has been used here.

The lower moisture in the curd of batch 6, as compared with 3 at the time of hooping, is probably associated with the higher acidity of the former group and the fact that, whereas batch 3 was hooped only 23 hr. after ladling, batch 6 remained for 45 hr. before being hooped. That more calcium is left in both groups which remained only a normal time in the whey is seen from the figures, which confirm those given tentatively for group 5, that about 50% of the calcium in the ripe Stilton cheese is in the coat which is removed before eating.

Samples of milk, curd and whey taken during the manufacture of the cheese were examined in the three remaining groups, and from these it is possible to calculate the amount of the calcium which is lost after the cheese is hooped, which will include the amounts (already given in the text for batches 2 and 4) in the drainings during the first 10–14 days after hooping. It is recorded for group 1 that the coats were thin with no wet

underlayer, and the distribution of the calcium between the coats and crumb is about equal. In batch 2 the coat of no. VIN was rather thick; the thickness is reflected in the relative weight of coat and crumb of this cheese. For batch 4, records show that the coats of all the normal cheese were moderately to very thick; in two cases the under-coat was also thick, but this was not the case in the other cheese of this group. This is also reflected in the distribution of the calcium in the cheese between the crumb and coat.

The weight of milk used in each set of four cheese is not of course known, as there was no separation until the curd was ladled, neither is it known in two cases whether the total original weight recorded includes or excludes very small quantities (about 0·0015%) which were removed for sampling, but a very close approximation to the right amount can be made for each batch, from which the total loss in each of the batches 1, 2 and 4 can be deduced.

Table 14

Batch no.	Wt. of Ca in milk (lb.)	Wt. in curd at hooping (lb.)	% lost	Wt. in ripe cheese (lb.)	% lost	Wt. with coats removed (lb.)	% lost
1	1·25	0·76	39·2	0·38	71·2	0·173	86·2
2	1·21	0·70	42·1	0·23	81·0	0·130	89·3
4	1·02	0·58	43·1	0·19	81·4	0·084	91·7

Table 15. *Distribution of sodium chloride between crumb and coat of cheese in group* 3

Normal or L.T.W.	Cheese no.	Wt. of crumb (lb.)	Wt. of salt (lb.)	% of salt	Wt. of coat (lb.)	Wt. of salt (lb.)	% of salt	Total wt. of crumb (lb.)	Total wt. of salt (lb.)	Total wt. of coats (lb.)	Total wt. of salt (lb.)
Normal	*IX*	7·21	0·22	2·99	3·28	0·086	2·63				
	X	7·66	0·24	3·14	2·41	0·063	2·60				
	XI	7·61	0·27	3·48	3·28	0·096	2·94				
	XII	7·51	0·23	3·00	2·69	0·069	2·57	29·99	0·96	11·66	0·31
L.T.W.	*IX*	7·31	0·23	3·14	3·04	0·071	2·36				
	X	6·82	0·21	3·08	3·14	0·074	2·35				
	XI	7·21	0·23	3·14	2·91	0·070	2·41				
	XII	7·76	0·24	3·14	2·55	0·057	2·24	29·10	0·91	11·64	0·27

It is shown from these calculations that, of the original calcium which is in the milk for the manufacture of Stilton cheese, approximately 40% is lost by the time the curd is hooped. Of the remainder, about 30% is lost during the first fortnight after hooping and by the time the cheese are ripe about 85–90% has disappeared from the edible part of the cheese.

It is also clear from Table 13 that less remains in those cheese which were allowed to stay in the whey for an abnormally long time and no compensating superiority in these cheese could be observed.

It may be queried whether this loss of mineral salt in the coat of Stilton cheese is more apparent than real, but a consideration of the relative weights of the crumb and coat shows that twenty-four cheese in three batches with a total weight of edible crumb of 186 lb., have 0·34 lb. calcium, whereas the coats of these same cheese with a weight of 57·5 lb. have a calcium content of 0·46 lb. A study of the acidity and moisture content of the curd at the time of salting so that a thin coat may result on ripening, or even the possibility of making a crustless Stilton-type cheese, would, it appears, be worth considering.

In addition to the analyses recorded in this paper, many others were made on these cheese, including total solids, fat, sodium chloride and nitrogen distribution. None of these

estimations was made on the coat except that in group 3 the sodium chloride was determined in crumb and coat separately. The figures are given in Table 15.

Approximately 60% of the salt which is added to the Stilton curd at the time of hooping remains in the ripe cheese before the coats have been removed. Of the amount left in the cheese, 24% is lost by the removal of the coats in the normal group and 23% in the L.T.W. group, and in each group more is left proportionately in the curd than in the coat. There is no accumulation of salt in the coat as with calcium.

REFERENCE

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REVIEWS OF THE PROGRESS OF DAIRY SCIENCE

SECTION D. NUTRITIVE VALUE OF MILK AND MILK PRODUCTS

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I. INTRODUCTION

The present review covers some $2\frac{1}{2}$ years, up to June 1950—a return to more normal conditions. Access to the literature has on the whole been easier, but as an aftermath of the war there has still been a steady trickle of papers published several years ago. The more important of these, together with some other papers omitted from the last review(1), are now included. We have endeavoured, as on previous occasions, to read papers in the original: those seen in abstract only are indicated by an asterisk in the bibliography. The selection of articles for the review was again

largely based on abstracts published in, or prepared for, *Dairy Science Abstracts*. We are very grateful to Mr W. G. Sutton, late Director of the Commonwealth Bureau of Dairy Science, to Mr A. W. Marsden, his successor, to Miss D. Knight, Librarian of the National Institute for Research in Dairying, and to their staffs, for their generous help which has greatly eased our task.

II. GENERAL

The twelfth International Dairy Congress was held after a lapse of 12 years. Its reports (2) contain much of interest to nutritionists. Those dealing with specific aspects are mentioned in the relevant sections of this review. Of books on milk of nutritional interest three, one new (3) and two in a new edition (4, 5) may be mentioned, and also Davis's (6) *Dictionary of Dairying*. Clements's book (6a) on infant nutrition contains a valuable chapter comparing the composition and properties of cow's and human milk. The 1948 year-book of the U.S. Department of Agriculture (7) deals mainly with grass, but has some valuable information on milk. Homb (8) reviews the value of pasture for the dairy cow. A useful précis of abstracts on milk published during 1949 will be found in (9). A National Research Council survey of the food and nutrition research in the United States during 1947 (10) and 1948–9 (11) lists much work in progress on milk, and the research programme of the U.S. Bureau of Dairy Industry (12) quotes projects of interest to nutritionists. A well-indexed bibliography of bulletins published by Agricultural Experiment Stations in the U.S.A. between 1881 and 1947 (13) should prove helpful. That part of a 'periodical manual of world literature on dairying' dealing with milk as a foodstuff contains many useful references, especially to German wartime publications otherwise difficult of access (14). Smith (15) and Krauss (16, 17) have reviewed work on the food value of milk and milk products, and papers (18, 19, 20, 21) also deal with this subject. Jeans (22) and Stevenson (23) have compared the composition of cow's milk with that of human milk. Several tables of food composition (24, 25, 26, 27), including one issued by the F.A.O. for international use (24), contain values for milk. Another, devoted specifically to the composition of milk and colostrum (28), should prove very useful: it lists values (mainly from the American literature) for a far greater number of individual components than any summary yet published. American official standards for the composition of milk products are given in (29). Breirem (30) has reviewed Norwegian work on the effects of the fodder on the compositional quality of milk. A review by Génin (31) of some recent developments in the dairy industry in several countries includes items of interest to nutritionists.

III. NUTRITIVE VALUE AS ESTIMATED IN LABORATORY AND FIELD EXPERIMENTS

A. RAW MILK (AND BUTTER FOR VITAMINS A, D AND E)

(*a*) *General*

Brief summaries of work at Coonoor, India, on different aspects of the nutritive value of milk will be found in (32). A progress report on food in old age by McCay (33) indicates that an exclusive diet of mineralized milk with cod-liver oil is as satisfactory for the rat as a mixed human diet. Addition of milk to a diet of mixed breakfast foods and vegetables (34), or to a poor (South Indian) diet (35, 36), improved the growth of rats and was beneficial to monkeys declining on the '10-in-1' American Army ration (37), but was of no value in improving lactation in rats receiving pork as a source of protein (38). In the outstanding experiments of Reyniers and his school (39, 40) and of Gustafsson (41) on germ-free rearing of animals, milk and its products and cream formed the basis of rearing mixtures for rats, whether germ-free or not, and were included in other diets. Milk is strongly protective against caries in the ordinary rat (42) and in the cotton rat (43), much more markedly so in the liquid than in the dry form (44). However, paper (45) seemingly contradicts both findings for the cotton rat. As judged by the performance of rats, milk from cows given fodders produced on depleted soil fertilized with nitrogen only was no different in nutritive value from that derived from fodders grown in limed fields heavily fertilized with phosphorus, potash,

nitrogen and trace elements(46). Other tests with rats showed that neither the toxic principle of cottonseed meal(47) nor thyroid-active substances(48) pass into the milk. Schulz(49) outlines the technique of animal experiments with milk and milk products in the laboratory of a German whey plant. Scott & Norris(49a) have compared, in experiments on rats, the nutritive value of human milk and colostrum with that of artificial mixtures of similar composition based on cow's milk.

An article by Mantillaro(50) discusses the composition and nutritive value of colostrum. The trypsin inhibitor of colostrum is at its peak on the 1st day after calving(51).

(*b*) *Vitamins*

(i) *General*

The first volume of a book on vitamin methods(52) describes physical, chemical and microbiological measurements of general application, but a specific method for milk is given only for vitamin A and carotene. An authoritative review of vitamins in milk published in 1945(53) was not mentioned in our previous report(1); that of Macy, Kelly & Sloan(28) contains a wealth of information. Greenbank(54) has discussed in detail the relation of oxidized flavours in milk to its constituents, including vitamins. Mouriquand(55) considers in a general way the vitamins of milk and certain milk products. A (non-specific) chronaximetric method is suggested for their estimation(56,57).

(ii) *Vitamin A and carotene*

Summaries of work on vitamin A previously done in Norway(30) and England(58) have appeared.

Methods of measurement. Several papers describe methods for the measurement of vitamin A and carotene in milk. The Carr-Price reaction was largely used for vitamin A, which was mostly assessed by measuring absorption visually or photometrically, or by direct colour-matching methods(59,60,61,62,63,64,65,66,67,68). Barnicoat(62), however, used a Lovibond tintometer, and preference for this instrument is indicated in paper (63). In this paper, and also in (62), factors are quoted for the conversion of Moore blue units to international units. The conversion factor applicable in our laboratory is given in (69). Those used by two groups of Indian workers are quoted in (70,71). The standardization of vitamin A quoted in (67) was based on 'Vogan', whose potency may have been seriously overestimated(72). According to (64), the same values were found for vitamin A, whether measurement was done with milk or with the fat extracted from it. Vitamin A in milk was also measured by ultra-violet absorption(63). A new sensitive colour reaction with activated 1:3-dichloro-2-propanol is described in (73); it measures both vitamin A and carotene, and is said to be simpler than, and as satisfactory as, the usual methods. Adverse comment on this procedure is recorded in (66). The method described in (65) was specially adapted for colostrum. Details of chromotographic separation of the alcohol and ester of vitamin A are given in (74).

Paper (75) describes a method for the preparation of the unsaponifiable residue of relatively large quantities of butter, suitable for the subsequent measurement of vitamin A and carotene. The recovery of vitamin A in the different procedures is discussed in (60,63,73).

Caution must still be exercised in comparing vitamin A values found by different observers, not only because of instrumental factors and difficulties but also because of lack of agreement on the factor relating vitamin A, in terms of weight, to international units (cf. (1)).

Colostrum. Further evidence has been published that vitamin A(65,76,77,78,79,80) and carotene(65,81), given during pregnancy, increase the concentration of these substances in colostrum, and Turk(82) has commented on the practical significance of the procedure. A preliminary account of the Shinfield findings(1) has also appeared(83). Vitamin A alcohol and vitamin A ester were equally active, and tocopherols had no influence on their assimilation or on the assimilation of vitamin A and carotene from ordinary rations(77). On the other hand, soya-bean lecithin is reported to have been highly effective in promoting the transfer of vitamin A(76). At parturition the colostrum of cows given vitamin A and lecithin contained twice as much vitamin A, but less carotene, than that of cows given vitamin A only. The effect disappeared after 1 week. Lecithin

had no influence on the vitamin A content of colostrum of cows given no supplement of vitamin A during pregnancy. In several instances the prepartum administration of large quantities of vitamin A depressed the carotene content of colostrum (65, 76, 79, 83). According to (65) the effect was less with vitamin A given after calving.

Information on the composition of colostrum under normal or experimental conditions comes from India (84, 85), New Zealand (62) and the United States (65, 81).

Whatever the prepartum feeding, observations now available support the earlier findings (1) that individual variations in vitamin A and carotene in colostrum are very marked (65, 77, 84) and that, on the whole, the colostrum of heifers is richer in these factors than that of cows (65, 83, 84, 85). According to (65) the concentration of carotene was higher for Jerseys and Guernseys than for Ayrshires or Friesians (Holsteins); the differences in vitamin A were not clear-cut. Although in India the differences were less marked (85), all workers naturally agree that colostrum is much richer in vitamin A and carotene than milk and that the rate of transition is rapid. According to (65) it is logarithmic. An interesting study of the composition of successive 2 lb. portions of the first milking after calving (78) of twenty-four cows indicates a rise in vitamin A and carotene associated with an increase in fat content. In the fat, the concentration of carotene decreased progressively, but that of vitamin A remained constant. This difference in behaviour between carotene and vitamin A is no doubt related to the increase in size of the fat globules with the successive milkings also observed by Eaton and his collaborators (Eaton, private communication). This phenomenon is discussed in (1).

The practice of starting milking before the calf is born is on the increase. In keeping with its general tendency to reduce the difference in composition between milk and 'colostrum' the procedure lowers the vitamin A and carotene content of the secretion produced after calving (78, 79).

Milk. Workers at the U.S. Bureau of Dairy Industry (86) have commented on the findings of the nation-wide survey of the vitamin A potency of milk and butter fully discussed in the previous review (1). Some further information, still incomplete, has appeared (58) about the survey done in this country in 1943–4.

Analyses of factory butters from two districts in New Zealand, done in 1935, have now been published (62). For total vitamin A activity a maximum of about 50 and a minimum of some 35 i.u./g. fat are quoted. The lowest values were obtained in late summer, probably because under the grassland-farming conditions of New Zealand the best grazing is available during the mild winter. A similar seasonal trend is reported for 1943–4 (87). A detailed Australian study of the vitamin A content of milk fat, based on examination of samples of butter or cheese taken at random from production in four dairying districts in Victoria in 1945 and again in 1947–8, is also now available (66). The similar dairying conditions in Victoria and in New Zealand are reflected in the similarity of the results. The highest values, 50–60 i.u./g., were obtained in winter, when green, slow-growing pastures are available. The summer values were about 30–40 i.u./g. Exceptionally high values observed during the 1945 'flush' of grass are attributed to a concentration effect due to a low production of milk. At all times the contribution of carotene to the potency of the fat, 35–50% of the total value, was markedly greater than that reported from Europe or the United States (1), though similar to that in New Zealand (62). The Australian workers relate the phenomenon to the carotene-rich diet of continuous pasture. Though this effect cannot be denied (1), the large proportion in both countries of Jerseys and of Jersey crosses may well have been the determining factor.

Values for market milk are available from Russia (88), the mean for eighteen samples during stall feeding being 5·9 μg. for carotene, and 93 μg./100 g. milk for vitamin A, and for forty pasture samples 17 and 300 μg./100 g., respectively. The values for vitamin A, like those previously quoted (1), seem improbably high.

Ray Sarkar (89) investigated, in India, the seasonal variations in vitamin A and carotene in the milk of a herd of Hariana cows; in winter the values for vitamin A were about 30 i.u./g. fat (calculated on the basis 1 i.u. = 0·25 μg.), and for carotene about 3 i.u.; in summer the corresponding values were 48 and 6. A few values for Indian fresh milk are given in (36). In Cuba, analyses of samples from Havana cafeterias (90) indicated much higher content of vitamin A and carotene in

'Grade A' than in 'Grade B' milk; the fat content is not stated and the difference may well have been due to differences in it. Six samples of milk in Singapore contained 113–167 i.u./100 ml. (91). Isolated figures for total activity of butter of 20 i.u./g. in winter and 50 i.u./g. in summer come from Germany (92). In France sixteen samples of milk at an unspecified time of the year contained 32·5–118 (mean 71·4) i.u./100 ml. (93).

Values for buffalo milk expressed in Moore blue units are available from India (70, 71). According to (71) a pigment, probably carotene, appears in the milk, usually colourless, of buffaloes when they receive an abundance of green fodder.

Krukovsky & Whiting (94) found a highly significant correlation between the tocopherol and carotene content of the milk of cows of four breeds at different seasons, but no correlation between tocopherol and vitamin A.

As in previous years, publications dealing specifically with the relation of feed to the vitamin A content of milk have been numerous. The effects of pasture (64, 66, 95, 96, 97), silage (64, 86, 98, 99) and hay (86, 97, 100) occupied much attention. The value of legumes, as pasture (96) or as hay (97), was again evident, and the superiority of silage over hay confirmed (86). The values for silage milk from Holland (99) are relatively low, but in keeping with other results from the same laboratory (cf. (1)). The value of carrots was studied in Germany (67, 101), and that of artificially dried lucerne [cake?] in Belgium (102). In the latter experiments some 450 mg. daily of the carotene from lucerne, added to a basal ration already supplying 940 mg. daily, had no effect on the potency of milk, which contained only some 10 μg. vitamin A and 11 μg. carotene per 100 ml. Pasture promptly increased these values to about 28 and 40 respectively. The authors suggest that the carotene was present in the lucerne in a non-available form. As would be expected, wheat in excessive amounts lowered the vitamin A potency of the milk (103). With their high carotene content, sweet potatoes proved a good source of milk vitamin A (104, 105). On the other hand, cottonseed depressed vitamin A in milk (106) and raw soya-beans its carotene content (107), probably by interference with the utilization of the carotene of the fodder.

Formulae relating carotene intake to the potency of butter come from Norway (64), and the United States (86, 108). The American authors (86) think that a cow needs about 480 mg. carotene daily to produce butter of average 'winter' potency, and 2500 mg. for the summer level. Consideration of feed-milk relationships in this connexion will also be found in (96). The proportion of carotene to vitamin A quoted in (97) is remarkably high for milk of Friesian cows. In the course of 19 months active carotenoids amounted on the average to 76% of the total in Guernsey milk and to 81% in Shorthorn milk (58). In 200 samples from bulk milk supplies the proportion rose from a mean of 62% to one of 80% as the total carotenoids increased from 1·7 to 11·0 μg./g. fat (58).

Using monozygous twins Winzenried & Wanntorp (68) studied the factors affecting the level of carotene and vitamin A in milk. They confirmed the well-known effects of feed and season, and found a decrease in vitamin A potency of milk fat towards the end of lactation. By eliminating external influences statistically, they were able to demonstrate that the vitamin A and carotene content and ratio in milk are genetically controlled. In studies with cows of the usual type no effects of stage of lactation were demonstrated (62, 89).

As far as we know, no papers on the effect of the administration of massive doses of vitamin A before calving, other than those quoted on p. 319, have been published. In some of these experiments (65, 76, 77) observations were carried beyond the colostral stage and, as would be expected, the increase in concentration of vitamin A persisted in the early milk. As with colostrum, tocopherols given before parturition had no effect (77). Tocopherols given during lactation were equally inactive (109). Vitamin A, in the moderate quantity of 100,000 i.u. daily, increased the concentration of vitamin A in the milk but did not depress the carotene (107). Cod-liver oil, at the rate of 5 oz. daily, depressed the fat content of the milk in the usual way (cf. (110)), and lowered the carotene and increased the vitamin A in the fat (109). Interruption of milking during established lactation was followed by a marked fall in milk yield, and a marked increase in concentration of vitamin A and carotene in both fat and milk (111). The milk of cows maintained at a temperature of 105° F. did not differ appreciably in carotene and vitamin A from that of cows similarly fed and kept at 50° F. (112). Administration o fiodinated protein had no effect on the content in milk

of carotene (48, 113); according to (113) vitamin A was also unchanged, but results in (48) indicate a reduction. A claim for *in vitro* conversion of carotene to vitamin A under the action of iodinated casein (114) has been contradicted in two laboratories (115, 116).

In Germany, carotene, rather than synthetic dyes, is recommended for colouring butter (92). Its value in this respect has been studied in detail by Richardson & Konar (117), who found that it had no deleterious effect on the keeping quality of butter in cold storage. Reinart (118), however, considers the native carotene of butter as one of its pro-oxidative constituents. The popular belief, in Holland, that carrots prevent the deterioration of salt-preserved butter has not been substantiated (119). In artificial ghee, according to Sadana & Ahmad (120), the stability of the crude carotene extracted from dried carrots by hot fat was not improved by adding α-tocopherol or ethyl gallate as anti-oxidants. Artificial butter colour, that had oxidized on keeping, caused the appearance of oily flavour in butter (121). Nordihydroguaiaretic acid added to milk increased the stability of vitamin A and carotene in the cream made from it (122). A patent (123) covers the use of 3-mercaptopropionic acid or ester for the prevention of oxidized flavours in milk. The losses of vitamin A and carotenoids from milk during the oxidative deterioration of milk fat are discussed in (124).

Vitamin A acetate proved more stable than a concentrate when used for the fortification of ghee (125). Paper (126) deals with the losses in vitamin A and carotene of ghee heated to temperatures of up to 250° C. Though large quantities of cottonseed in the ration depress the vitamin A content of milk (p. 321), the stability of vitamin A and carotene in ghee made from such milk appears to to enhanced (106). Vitamin A was stable in ghee containing 10% free fatty acids, but diminished rapidly in rancid ghee (127).

(iii) *The vitamin B complex*

General. With the great development of microbiological methods, rapid and relatively simple procedures are available for the determination of most members of the vitamin B complex. Though useful and reliable in the hands of specialists, such procedures present many pitfalls for the unwary, and their hasty application without proper checks for the assessment of nutritive factors essential for higher animals has brought much confusion into an already complicated field.

Measurements by microbiological methods of several vitamins in milk are described in (128). The tests, though published in 1949, were done nearly 10 years ago when the technique was still in a very experimental stage.

Vitamin B_1. For eight samples from a Paris dairy, values are quoted of 34–63 μg./100 ml. (mean 52 μg./100 ml.) (129). Milk produced by cows receiving large quantities of silage (130) had the same content of free vitamin B_1, 20–30 μg./100 ml., as milk of cows given only hay and concentrates (128). About half of the total vitamin B_1 was in a bound form which could not be satisfactorily measured (128). In the experience of Hibbs & Krauss (48) iodinated protein depressed the total vitamin B_1 content of milk, but Bartlett, Rowland & Thompson (113) observed a decrease only in the free, unphosphorylated, vitamin related to the well-known decrease in milk phosphatase.

Riboflavin. Hoffman (131) has reviewed fluorimetric methods for the determination of riboflavin in milk. The content of twelve samples from a Paris dairy varied from 57 to 161 μg./100 ml., with a mean of 87 μg./100 ml. (129). Jenness & Coulter (132) made an interesting study of substances in milk and milk products fluorescing in ultra-violet light. Mean values for some fifty samples each of factory milk and market milk in Russia are very low, 74 and 57 μg./100 ml. respectively (133) (see also p. 332). That the adequacy of methods should be checked by biological assay was shown by the observation that a procedure satisfactory for the fluorimetric measurement of riboflavin in cow's milk was unsuitable for sow's milk (134). Silage, 15–20 kg. daily, had no effect on the vitamin B_2 (presumably riboflavin) content of milk (135). Preliminary reports have appeared of a study in England of the destruction of riboflavin in milk by light during distribution (136, 137). Observations from Russia (133) indicate losses of 15–30% on exposure of factory and market milk to light in clear bottles for 24 hr. For experimental purposes 96% of the milk riboflavin could be removed by irradiation (138). The riboflavin content of milk was slightly reduced in hyperthyroidism resulting from administration of iodinated protein (48, 113).

Nicotinic acid. Only one report on this vitamin has come to our notice (128); the author, however, makes it clear that the analytical method used may have been open to question.

Pantothenic acid. The values, by a microbiological method (139), for two samples of milk of 1·3 and 2·5 mg./100 g. are much higher than those usually obtained by such methods (cf. (1)). According to (128) the pantothenic acid content of milk declines with the progress of lactation.

Vitamin B_6 (pyridoxin). Two valuable papers from Snell's laboratory deal with the microbiological measurement of pyridoxal (140) and with the distribution of the three forms of vitamin B_6, pyridoxal, pyridoxamine and pyridoxin, in natural products (141). For fresh, whole milk mean values of 32 and 9 μg./100 g. were found for the first two components (expressed as the mono- and the di-hydrochloride respectively), but no pyridoxin as such was detected.

Biotin. The only values noted are 2·0–2·5 μg./100 ml. for whole or skim milk in Switzerland; they were obtained by careful microbiological assay (142).

Choline. Waugh, Hauge & King (143) studied, by a chemical method, the concentration of choline chloride in the colostrum and milk of five cows. At the first milking the mean content was 69 mg./ml. It declined rapidly to 17 mg./100 ml. at the sixth milking, and then slowly to 13 mg./100 ml. 4 weeks after calving. Povoli's (144) values, also obtained chemically, for Argentinian whole milk are 12–14 mg./100 ml.

Folic acid. Hodson (145) investigated critically the method for the assay of folic acid in milk. He obtained conflicting results by two microbiological methods and a chick assay, and concluded that the methods available were not suitable for milk though they all indicated that it was low in folic acid. According to (146) folic acid improved neither the growth of, nor the haemoglobin formation in, young rats on mineralized milk diets.

p-*Aminobenzoic acid.* The observations of Möller (128) do not confirm those of Scheurer, quoted in the previous review (1), that the feeding of silage markedly reduces the concentration of the factor in milk.

Vitamin B_{12}. Further work by Hartman & Cary (cf. (1)), still published largely in a preliminary form, indicates that the growth factor X present in fresh milk is identical with, or closely related to, vitamin B_{12} (147, 148, 149, 150, 151). The factor is apparently synthesized by the cow, since it was present in similar concentration in the milk of cows on pasture and in that of cows given, for a period of years, feeds devoid of vitamin B_{12} activity (149, 151). According to Hartman, Dryden & Cary (151) the content in milk measured biologically is about 1·2 μg./100 ml. However, Collins, Dietrich & Elvehjem (146) report, also by a biological method, the much lower values of 0·2–0·3 μg./100 ml. (see also (152)). Addition of vitamin B_{12} to a diet of mineralized milk influenced neither the growth nor the haemoglobin formation of young rats, but addition of vitamin C increased markedly the vitamin B_{12} content of their livers (146).

Other factors. Anderson & Hogan (153, 154) postulate the presence in milk of an unrecognized vitamin different from vitamin B_{12}. Fuller reports have appeared of the work on unidentified factors (cf. (1)), also present in milk, needed by foxes (155) and minks (156). Milk (157) and raw cream (158) restored to normal the level of xanthine oxidase in the livers of rats given a purified diet, whereas known crystalline vitamins including vitamin B_{12} were ineffective; xanthopterin gave some positive but erratic effects (157).

(iv) *Vitamin C*

Values for milk—some raw, others unspecified—come from the United States (159), France (160), Italy (161), Finland (162), Norway (30) and India (163). Papers (161, 162) quote values for samples from individual cows. Those in (162) are surprising because of the large proportion of dehydroascorbic acid apparently observed immediately after milking. The method used in (160) has been adversely criticized (164). In India the milk of Tharparkar and crossbred cows contained less vitamin C (some 2 mg./100 ml.) than that of Sindhi (2·4 mg./100 ml.) and Gir (2·2 mg./100 ml.) cows, and of Murrah buffaloes (2·6 mg./100 ml.) (163). Similar breed differences were noted in the previous review (1). Values observed during the first month of lactation were lower than those found later (163).

Neither the injection of thyroxine (119) nor the administration of 'Protamone' (an iodinated casein) (48) to cows lowered the ascorbic-acid content of the milk, but this was depressed by

treatments involving the consumption by the cow of free iodine (113). The conflicting results mentioned in the previous review (1) may, therefore, have been due to the presence of free iodine in some of the preparations of iodinated protein.

The relation of vitamin C to oxidized flavours has been further discussed in interesting publications, including a review of work done in Holland during the German occupation of the last war (165). The milk used in several of these studies had been subjected to some form of heat treatment, generally pasteurization and also homogenization, but it will be simplest to consider in one place all the work on this subject. It was pointed out in the previous review (1) that, though ascorbic acid added in excess exerts a protective action, the quantity naturally present, especially after partial photo-oxidation, may actually promote the development of the taint which can be prevented by complete removal of the vitamin. Recent evidence supports these observations. Thus, papers (166, 167, 168, 169, 170, 171, 172) indicate that the addition of ascorbic acid to milk to the extent of 2–12 mg./100 ml. retarded the appearance of oxidized flavours and increased the keeping quality of the milk even in the presence of added copper (166). De-aeration of the milk afforded further protection (170). With cream, added ascorbic acid was much more effective than hydroquinone in preventing the development of metallic flavour (173). Work described in (168, 171), and that from Krukovsky's laboratory (124, 174), confirms the protective effect of complete removal of the vitamin, and that in (124, 171, 174) the untoward effect of exposure to light. During storage in the refrigerator the added ascorbic acid (166, 168), and that present naturally (175, 176), decreased rapidly owing to persistence of a high oxidation-reduction potential (176). In frozen milk (168, 170, 172) the loss was slower, the rate diminishing with temperature (170) and with increasing initial concentration of added vitamin C (172). At room temperature the initial rapid drop was checked when bacterial growth lowered the *Eh* (176). According to (177), where other information on stability of vitamin C in milk will also be found, addition of 0·01 % thiourea to milk contaminated with copper retarded losses of vitamin C on storage at 5° C. Holmes (178) found a decrease in reduced vitamin C from 1·8 to 0·6 mg./100 ml. during storage of cow's milk in darkness at 10° C. for 5 days. Addition of riboflavin, 0·4 or 0·8 mg./100 ml., had no effect on the rate of loss (see also p. 327).

Crowe, Jenness & Coulter (179) studied the reducing capacity of milk by the ferricyanide procedure. Ascorbic acid contributed only about a quarter of it. According to Neuweiler (180), cow's milk lacks the protective substance against the ascorbic-acid oxidase of cucumber said to be present in human milk.

A study of the photochemical destruction of vitamin C in milk has been briefly reported (181). The effect of light was most marked at pH 6·5 and the rate of oxidation increased by 35 % for a 10° (C.?) rise (see also p. 329).

In a study of the effects of different stainless steels on the vitamin C of milk the destruction proved greater with manganese steel than with titanium steel; nickel and chromium steels were inactive (182, 183).

(v) *Vitamin D*

The tentative biological method of the Association of Official Agricultural Chemists for vitamin D in milk (184) has been recommended for official adoption (185).

A chemical method for milk and milk products based on the measurement of the colour given by vitamin D with antimony trichloride in the presence of guaiacol has been described (186, 187, 188, 188*a*, 189) (see also p. 328).

Surprisingly high values of 6–18 i.u./g. butter or ghee, measured with rats on the non-saponifiable residue (cf. (190)), and of 32 i.u./g. for a similar measurement on butter itself, are reported from India (191). The high values are attributed to the strength of the sun to which Indian cows and their fodder are exposed.

(vi) *Vitamin E*

Values by chemical analysis for the vitamin E content of milk or butter are given in (94, 97, 192, 193). They are, in general, about 20–40 μg./g. fat, though in the most extensive series of measurements,

that from Sweden (192), the extremes were 6 and 45 μg./g. Much of this variation was due to seasonal effects, values in late summer and autumn being 2–2·5 times higher than in winter and spring. Smaller seasonal effects were observed in America (94). As can be seen from (97) the seasonal effect was most probably connected with the feed. No obvious differences were noted in the concentration of vitamin E in the milk of Friesian, Brown Swiss, Guernsey and Jersey cows (94). Administration of tocopherol increased the tocopherol content of butterfat (82, 109), but 5 oz. daily of cod-liver oil had a depressing effect (109). Cod-liver oil also lowers the blood level of vitamin E in the cow (194). According to (97) milk with a tocopherol content of less than 20 μg./g. fat readily develops oxidized flavour. Reinart (192) doubts whether tocopherols are ever present in butter in sufficient concentration to exert anti-oxidative effects. In his experience summer butter, relatively rich in tocopherols, was more prone to oxidized flavours than winter butter. Added tocopherol afforded only moderate protection. For frozen milk, abstract (169) agrees with this latter observation, but very definite protection is briefly reported in (82). Nordihydroguaiaretic acid protects vitamin E in stored cream from destruction (122), and is more effective than added tocopherol in improving the stability of frozen milk (169). Losses in the course of the oxidative deterioration of milk fat are considered in (124).

(c) *Other factors*

(i) *Minerals*

A new edition of a bibliography of minor elements has been published (195); its usefulness is diminished by an inadequate index. Waite (196) has reviewed information on heavy metals in milk.

A further contribution to the Canadian survey of the calcium and phosphorus content of milk from different districts has appeared from Montreal (197), the values for the raw, pasteurized and homogenized product being 0·112–0·115% for calcium, and 0·089% for phosphorus. The very high value of 0·1526% calcium is reported from India for a composite sample from ten Sindhi cows, and a value of 0·1910 for a similar sample from ten Murrah buffaloes (198). Work in Norway (30) disclosed no effect on the calcium content of milk of an increase in calcium intake. Shortage of milk in India has led to the study there of the value of vegetable substitutes from groundnut (199) and soya-bean (200) supplemented with calcium.

Values for the iron content of milk are available from the U.S.A. (201, 202), Belgium (203, 204) and England (205). The (mean) values are within the range 0·3–0·7 mg./l. As would be expected, the lowest concentrations were in milk that had been handled but little (201); with this milk, in observations lasting from January to May, the lowest values were obtained in March and April.

Iron in butter was measured in Belgium (206), Holland (207, 208), Finland (209) and New Zealand (210). The range of concentrations was great and even means of large numbers of determinations differed markedly, e.g. the Dutch national mean was 0·65 μg./g. (207), the Belgian value 1·5 μg./g. (206) and the Finnish one, where the content had increased as a result of the last war, 2·3 μg./g. (209). The fluctuations and the high concentration encountered are, naturally, largely due to adventitious iron; according to (207) the creamery water supply is the most important source of contamination.

Dahl (211), in Denmark, found no measurable copper in four samples of milk taken directly from the udder in winter, but 0·12–0·15 mg./l. in commercial milk. For similar milk the German value (212) is about 0·25 mg./l.

Values for copper in butter are available from the U.S.A. (213), New Zealand ((210), and for cream (214)), Belgium (206), Holland (208), Denmark (215), Finland (209) and Germany (212). The values are extremely variable, e.g. the range for Belgian samples (206) was 0·05–0·95 μg./g. with a mean of 0·3 μg./g.; the contamination there was rather higher in winter than in summer. The content was inversely related to the grade of the butter (206, 215). With butters badly contaminated with iron and copper 'A.I.V. Salt' exerted a protective action (209).

Paulais (216) found in milk some 0·5 μg./l. of cobalt. It is of interest that nickel, though chemically closely related to cobalt, is neither a natural constituent of milk nor appears in it when given in large quantities to the cow (217). The German workers (212) found no lead in milk or butter, and zinc to the extent of about 1 mg./l. and 1·7 μg./g. In Holland, manganese in butter amounted to some 0·06 μg./g. (208).

McClure (218) has reviewed recent information on the relation of fluorine in milk to that taken by the cow. Administration of iodinated protein increases the iodine content of milk tenfold (219) to one-hundred-fold (113).

(ii) *Proteins*

We have remarked in the previous review (1) on the surge in the study of proteins and amino-acids in nutrition. Progress has continued, and valuable additions have been made to this aspect of the knowledge of milk as food. A useful review of published work is given in a book edited by Sahyun (219*a*).

For the proteins of freeze-dried milk at an 8% level of intake Henry, Kon & Mawson (220) give a biological value of 85 with a true digestibility of 93. McMeekin & Polis (221) have collated the information about the proteins of milk, and data about its amino-acid composition are given in (222, 223, 224, 225). An exhaustive article on methionine (226) contains useful data on its presence in milk and milk products. The proteins of milk are richer in methionine and isoleucine but poorer in threonine and arginine than those of colostrum (224, 225).

The 'immune proteins' of colostrum and the globulins of milk have been analysed (227, 228). Block & Bolling (229) determined the amino-acids of the non-protein fraction of milk and found, as with proteins, a great similarity in this respect between cow's and human milk.

It is accepted that proteins of different amino-acid composition have the same biological value for the adult ruminant, and this is taken as evidence of the degradative and synthetic activity of the micro-organisms of the rumen. Experimental support comes from the work of Block & Stekol (230), who demonstrated the passage of labelled sulphur given to a cow as inorganic sulphate into the molecules of cystine and methionine in her milk.

The contribution of the proteins to the fluorescence of various milk fractions in ultra-violet light is discussed in (132).

(iii) *Fat*

In new editions of his books (231, 231*a*) and in a review (232) Hilditch discusses authoritatively the composition of milk fat.

The value of milk fat continues to command attention, especially in relation to vegetable fats. The balance of evidence now seems to be against the possession by milk fat of special nutritive properties ((233, 234, 235, 236, 237, 238, 239, 240); see also (32)), and the same opinion is expressed by reviewers in Scotland (241) and in Holland (242) who are not involved in the controversy. A statement of the case by Deuel will be found in (243). The Wisconsin workers still believe in the superiority of butter when lactose is the carbohydrate in the diet (244, 245). They have confirmed (245) their earlier findings (cf. (1)) of lack of difference in the presence of other carbohydrates. They point out now, however, that the relative merits of butter and other fats vary according to their level in the diet (246), and the findings from another laboratory (247) agree with this view. Jansen and his collaborators remain of the opinion that summer-pasture butter possesses a value not shared by other fats (248, 249), but papers (233, 235, 236, 238, 239, 240) specifically deny this, and work reported in (250) discloses no superiority of milk fat over lard. Whatever the outcome of this controversy, there is now complete unanimity that vaccenic acid is in no way involved, being quite inactive (233, 235, 238, 240, 245, 248, 249, 251, 252). Moreover, Δ^{12}-octadecenoic acid has no growth-promoting value (235). It should be noted that rape-seed oil, one of the fats tested by the Dutch workers ((248, 249, 252); cf. also (1)), was found inferior in other laboratories to fats other than butter (233, 251), probably because of its abnormally low digestibility (253). Tests at Shinfield showed no difference between summer butter either from New Zealand or from local Shorthorn or Jersey cows (254), although previous work (240) had indicated the superiority of the New Zealand butter, but not of the local butter, to arachis oil.

Evidence continues to accumulate that the liquid, more unsaturated fraction of butterfat produces better growth than the other fractions (255, 256, 257) or butterfat itself (258).

The Wisconsin workers (259) now think that fat affects the utilization of galactose only indirectly by slowing up its absorption, a view supported by the interesting experiments of Richter (260)

which, however, show that the reverse influence, that of galactose on the metabolism of fat, may be profound.

According to De & Karkun(261) ghee is superior to coconut oil. The interpretation of their results is, however, impossible owing to the marked difference in the levels at which the two fats were included in the experimental diets.

Cow and buffalo ghee were absorbed by rats at the same rate as sesame and coconut oils, and better than a number of other oils and fats tested(262). Their digestibility was progressively lowered by heating to temperatures up to 300° C.(263).

Butter promoted better retention of calcium and phosphorus by young rats than 'Dalda', a hydrogenated vegetable fat, both in the presence and in the absence of added vitamins A and D as cod-liver oil; growth was also rather better with butter(264). However, according to a brief report ((32), p. 43 there) rats grew and reproduced better with 'Dalda' than with butter.

Mildly oxidized butter(255) and rancid ghee(127) were not much inferior to the fresh products in promoting the growth of rats, but the ghee interfered with reproduction and lactation.

In self-selection studies young rats preferred hydrogenated vegetable oils to butterfat and untreated oils(265).

Fats low in unsaturated fatty acids, such as 'Crisco' and butter, retarded hepatic injury on low-protein diets(266). Butterfat added to a 'poor South Indian diet' did not improve its value for rats(35) (cf. (1)).

A detailed study of the cholesterol content of milk and its relation to season and breed comes from the United States(267). The mean value of 11·4 mg./100 ml. agrees well with that from Portugal(268) of 14 mg./100 g.

B. TREATED MILK

(a) *Heated milk*

(i) *General*

Work from India showed that neither pasteurization at 145 and 150° F. for 30 min. and at 162° F., for 5 min.(269), nor boiling and simmering for 5(269) or 10 min.(270), affected the total nutritive value of milk as judged by the exclusive feeding to rats of the mineralized product. Buffalo milk was equally unaffected by boiling(270).

Forti(271,272) has continued his work (cf. (1)) on raw and heated (pasteurized or boiled) milk as an exclusive diet for rats. Mouriquand(55) considers in a general way the effects of heat treatment on some of the milk vitamins.

(ii) *Vitamins*

Vitamin A and carotene. Boiling of milk for 3 min. had little effect on vitamin A and carotene(88). In Cuba, boiled milk contained more vitamin A and carotene than samples of raw and pasteurized milk obtained from different sources(90). The difference is attributed to loss of water during boiling. Pasteurization at 76·6° C. for 30 min. was more effective than that at 61·6° C. in retarding the oxidative deterioration of the fat-soluble vitamins of milk ((124), cf. p. 322). Further evidence of the protective effect of high-temperature treatment will be found in (175).

Riboflavin and nicotinic acid. The riboflavin of pasteurized milk was quite stable during storage in the refrigerator for up to 31 days, even when the milk was occasionally exposed to the light during sampling(175). However, exposure for 2 hr. to low, early morning, autumn sunshine in New Zealand in the course of delivery of pasteurized milk caused a loss of some 50–60% of the riboflavin(273).

Wartime values from Italy of 150–260 μg./100 ml. for nicotinic acid, only now available(274), are high by present-day standards (cf. (1)).

Vitamin C. Holmes(275) found a loss of 12% in reduced ascorbic acid in holder pasteurization. On storage in darkness at 10° C. for 4 days the pasteurized milk lost 77% of its vitamin C and the raw milk 64%. Addition of riboflavin(276) or of lactose(277) had no effect on the rate of loss. Losses after pasteurization by different methods were examined by Maxa(278), who found the smallest effect with enclosed plate-type pasteurizers. According to Randoin & Perroteau(160) boiling for 30 min. reduced by one-third the vitamin C content of raw or pasteurized milk;

autoclaving resulted in the loss of about one-half. Under the conditions of delivery, described above for riboflavin, pasteurized milk in New Zealand lost 50–60% of vitamin C(273).

Bernhart & Linden(279) studied the effect of heat treatment on the copper-induced oxidation of milk. High-temperature treatment reduced the pro-oxidative effect (cf.(122,175)). Tests with the stainless steels mentioned on p. 324 were also done at pasteurizing temperatures and at boiling-point, with similar but more pronounced effects(182,183). According to Maxa(278), stainless steel, aluminium and tin had no effect on the vitamin C of milk, but zinc, iron, lead, copper and brass caused losses. These were more marked with the first three metals at boiling-point than at 5° C.; with copper and brass the effect was reversed. Vitolin & Hesselvik(280) have measured the vitamin C content of milk mixtures for babies. Mouriquand & Stupp(281) found refrigeration the best method of preserving the reduced vitamin C in sterilized milk.

Vitamin E. Milk given to female rats with a diet deficient in vitamin E, to the extent of 1·5–3·0 ml. per 10 g. live weight, failed to prevent resorption of the foetuses(282). Boiled milk was, if anything, superior to raw.

(iii) *Proteins*

Jacquot, Matet & Fridenson(283) have reviewed the effects of heating on the value of milk proteins. For what they are worth, *in vitro* tests with soya-bean milk indicated that its proteins were only one-fifth as digestible as those of pasteurized milk(284). The authors themselves point out that the findings are at variance with those of *in vivo* tests previously done in their laboratory(285).

(*b*) *Irradiated and vitaminized milk* (*all types*)

The (U.S.A.) National Research Council(286) and the American Medical Association(287) consider the fortification of milk with vitamin A unnecessary, though the medical body does not object to its incidental addition in fish-liver oil concentrates used primarily to supply vitamin D.

Methods of increasing the vitamin A content of milk through the cow are considered in (288).

The irradiation of milk has long been used in Germany for the prophylaxis of rickets. Two distinct methods of irradiation are now available there, and there is lively controversy as to their merits. The old-established procedure of Scheer(289,290,291,292,293) uses high-pressure mercury-vapour lamps to irradiate a thin flowing film of milk. In that of Lembke(292,293,294,295,296) milk flowing through quartz tubes is irradiated by means of low-pressure burners. Over 95% of their radiations are at 2537 A. and, therefore, highly bactericidal, and the process is intended not only to produce vitamin D but to pasteurize the milk as well. Other advantages, including higher vitamin D potency, are claimed for it (294,295,296) but not necessarily conceded (290,292,293); it should be pointed out that irradiation by Scheer's method also improves the keeping quality of milk(297). The method of Scheer has already given good results in practice (cf. (1) and p. 341); that of Lembke has not yet been so tested. Quite recently an apparatus, in many ways similar to that of Lembke and manufactured by the same firm, has been described by Sauter(298). Much the same vitamin D content, about 1200 i.u./l., is quoted for the Scheer milk(189,290) and for that of Lembke(294,295), and Diemair & Manderscheid(189) quote a similar content for Swedish irradiated milk. These are very high values, and one might wish that the specificity of the chemical method used by Scheer(186,187,188,189) was more firmly established or that the biological tests supporting Lembke's claims(295) were more convincing. In fact, Auhagen & Grab(299) were unable to find by tests on rats anything like this potency in German irradiated milk, and express the opinion that the chemical method of Diemair & Manderscheid(188a, 189) is non-specific and gives values several times too high.

Auhagen & Grab(299) and other workers in Germany(300,301,302) now advocate the addition to milk of vitamin D_2 or D_3 as preferable to irradiation. According to these authors a 0·5% solution of the vitamin in ethyl alcohol can be easily dispersed in milk without emulsifying agents. Auhagen, Huf & Grab(303) state that vitamin D so dispersed in milk is no more active for the rat than in oily solution. It is present in the aqueous phase attached to the protein and follows it in the process of separation, whereas the vitamin D of irradiated milk is recovered in the fat. A discussion of the relative merits, in Germany, of irradiation and supplementation will be found in(304,305).

In the United States addition of vitamin D to milk to the extent of 400 U.S.P. units per (American) quart rather than irradiation is now the method of choice. The policy of the American Medical Association concerning this milk is stated in (287,306) and that of the (U.S.A.) National Research Council in (286). The views of the inspectors charged with the control of vitamin D milk in American cities will be found in (307). The results of an inquiry in eighty-eight cities and twenty-six states are quoted, according to which about 5% of the samples tested there proved to be below the stipulated potency. Addition of vitamin D to milk is also considered in (288).

A Federal specification for evaporated milk with added vitamin D will be found in (308), and the addition of vitamin D to milk in Sweden is mentioned in (309). According to (307), in many American cities vitamin D milk constitutes 30–60% of the total fluid milk sold, and Jeans (310) states that nearly all evaporated milk in the United States has vitamin D added to it. Paper (311) describes the manufacture, in this country, of evaporated milk fortified with vitamin D and gives general information about it.

According to Boisselot & Causeret irradiation of milk destroys about 16% of its vitamin B_1 (129) and some 10% of the riboflavin (129), but affects neither vitamin A nor carotene (93). Mouriquand (55) considers in general terms irradiation of milk to increase its vitamin D. An apparatus for pasteurization with ultra-violet light is described by Nicholson (312).

The addition of vitamin C to market milk, primarily to prevent oxidative taints, is advocated in (166,168,171,172). Holmes & Jones (313) studied the loss of vitamin C added to the extent of 75 or 150 mg./l. from milk kept at 10° C. in darkness.

A dried-milk product for babies, 'Similac', fortified with vitamin C, contained 66 mg./100 g. reduced, and 71 mg./100 g. total, ascorbic acid. The loss in gas pack was negligible—less than 3% in 1 year. From the open tin the loss was about 3% in 4 days and about 6% in 8 days. The loss after reconstitution was some 12% after 24 hr. (314).

Weckel (315) discusses in detail the value and composition of skim milk, and favours the addition of vitamins A and D to it; the measure is said to have the support of the American Medical Milk Commission.

(*c*) *Soured milk*

In a comparison of fresh milk and of soured milk (dahi) made from it, Sundararajan (36) found no difference in the way they improved a poor rice diet. When tested as an exclusive diet for rats, dahi, supplemented with minerals, proved slightly inferior to the original milk boiled (316). The difference is attributed to loss of some essential amino-acids in the course of fermentation. Paper (36) gives no support to this supposition. According to it souring did not lower the biological value of milk proteins. In experiments with dogs kefir caused greater secretion of saliva and digestive juices than milk (317).

Further contributions from South Africa deal with lesions of the pancreas (318,319), other organs and the teeth (318) of rats given the staple Bantu diet of maize meal and 'sour milk'. It should be pointed out (cf. (1)) that the 'soured milk' is, in fact, only the curd with the whey removed.

Papers (36,319) indicate some loss of vitamin A in souring. Riboflavin does not seem to be affected (133). The stability of vitamin C in milk is improved by souring; moreover, the dehydro-ascorbic acid present is largely reduced to ascorbic acid (176). Maxa (278) studied the losses from yoghurt of vitamin C originally present or added, including those due to exposure to light.

The availability of milk calcium to rats was not changed by souring (36).

When kefir grains or milk supplied 9–10% protein in diets offered without stint, rats grew almost as well with the grains as with milk (320). The value of soured milk for chicks is discussed in (321). Möllgaard (322) describes his special culture of lactic-acid bacteria (cf. (1)), and preliminary mention of experiments with it is made in (323).

(*d*) *Soft-curd milk*

In tests with rats milk treated with sodium hexametaphosphate given alone left the stomach more rapidly than ordinary milk; however, the rats grew and retained calcium and phosphorus equally well with both milks (324).

(e) *Milk treated by other methods*

Trout (325) has authoritatively reviewed work on the nutritive value of homogenized milk. The development and prevention of oxidized flavours in homogenized milk is discussed in (174). As would be expected, preservation of milk with hydrogen peroxide destroys its vitamin C (326). The possibilities of freeze-drying of milk are considered in (327).

C. MILK PRODUCTS

(a) *Cheese, whey and buttermilk*

(i) *Cheese*

General information about various kinds of cheese available in the United States, including that on nutritive value, is given in (328).

Vitamins. French values (329) for riboflavin are of the order of 300–400 for hard cheese and 700–900 μg./100 g. for Roquefort, Brie, Camembert and Bleu d'Auvergne. In the last two and in Gruyère there was no difference in content between rind and core.

For nicotinic acid the values varied markedly with type of cheese, being 45 for Gruyère and 700–2300 μg./100 g. for Camembert. With this cheese the rind contained much more than the core. Of pantothenic acid hard cheese contained 200–300, Roquefort 550–700 and Camembert 90–1000 μg./100 g., and again the difference between core and rind was much more marked for the last than for Gruyère or Bleu d'Auvergne. A value of 500 μg./100 g. is given for two samples of cheese in Denmark (139).

Ritter (142) measured biotin in fourteen varieties of cheese. The lowest value was for Emmenthaler, 0·04 μg./100 g., the highest, 20 μg./100 g., was for Limburger; full-fat Tilsit had 1·5, Gorgonzola 1·9, curd cheese 2·8, Roquefort 3·6 and Brie 8 μg./100 g. The outside of the majority of cheeses contained more biotin than the core, and the difference was most marked for Emmenthaler (5·2 and 0·02 μg./100 g. respectively) and for Tilsit (7·6 and 0·56 μg./100 g.), and was reversed for Limburger (11 and 14 μg./100 g.) and Brie (9·6 and 11·2 μg./100 g.). Ritter investigated further the surface synthesis of biotin (cf. (1)) by studying the production of biotin by micro-organisms obtained from the rind (330).

For 'folic acid' a value of 5 μg./100 g. is quoted for American Cheddar (331).

In the Argentine, determinations of choline gave 22 mg./100 g. for Parmesan and 47 mg./100 g. for processed Gruyère (144).

The rapid disappearance of vitamin C during cheesemaking noted in the previous review (1) has been confirmed (278).

American cheese contains, according to (193), 1 mg./100 g. of vitamin E.

Minerals. In Scandinavian countries whey cheese is considered as a valuable source of iron. Blix (332) points out that the metal is purely adventitious, coming from iron pots in the old method of preparation. Made in aluminium, copper or stainless steel vessels whey cheese was quite low in iron. Information is available about copper (196,212), iron (196), lead (212) and zinc (212,333,334,335) in cheese. Various cheeses contained from 44 to 180 (196) and from 40 to 230 μg./100 g. (212) of copper. No lead was found in cheese (212). For zinc, values of 230–670 (212), 2200 (333) and 950 and 1360 μg./100 g. (334) are given. Cheeses made in zinc moulds contained more (333,335).

Proteins. Block (336) describes interesting experiments with rats on the value of small quantities of cheese and other sources of protein of high lysine content in improving diets eaten by poor people in the United States. Analysis of American cheese for ten essential amino-acids is given in (222). Papers (337,338,339) deal with the contribution of amino-acids to the flavour of cheese.

(ii) *Whey (liquid and dried)*

Webb & Whittier (340) have written a most useful review on the utilization of whey, including its use in foods and feeds and for the manufacture of riboflavin by fermentation processes. From Holland, where there is a large surplus of whey, come papers on its utilization in bread-making (341) and on its improvement as a food product by electrolytic removal of salts (342). The work of

Daniel & Harvey (343) is of interest in this connexion; they found that addition of whey powder to a diet containing dried whole milk depressed the growth of rats, but that the opposite effect was obtained with dialysed whey; the inorganic constituents of whey apparently interfered with growth. A U.S. Department of Agriculture leaflet (344) deals with the use of whey in home cooking.

Whey and dried whey contain 2·4 and 40 μg. biotin/100 g. respectively (142). The choline content of three samples of dried whey was 2·13–2·36 mg./100 g. with a mean of 2·31 (345).

The unidentified factor needed by monkeys (cf. (1)) was present in the whey fraction, but drying apparently destroyed it as commercial dried whey was inactive (346) (see also (347)). Further reports on the presence in whey of unidentified factors for chicks will be found in (348,349,350,351,352).

Values for amino-acids in whey are given in (223,353).

In twelve samples of whey for infant feeding (wrongly described as *babeurre*) the high mean value for iron of 465 μg./100 g. (limits 180–735 μg./100 g.) is attributed to contamination in manufacture (204). An experiment on the retention of calcium from whey by adult rats is reported in (354).

(iii) *Buttermilk* (*liquid and dried*)

According to biological tests with rats and guinea-pigs dried buttermilk contains, as one would expect, neither vitamin A nor vitamin C. It is more surprising that it should also have been found deficient, by tests with pigeons, in vitamin B_1 (355). Russian buttermilk (liquid) contained 48–120 μg./100 g. of riboflavin (133). Roine & Csáky (356) found no difference between 'cultured buttermilk' and fresh whole milk as sources of all vitamins for the rat (it is not clear from the paper whether this was in reality buttermilk and not whole, soured milk). The basal diet, offered without stint, consisted of 80% potato starch, casein, vitamin-free margarine and salts. It should be pointed out that, since the potato starch was raw (Roine, private communication), refection, with synthesis of vitamins of the B-complex, might well have occurred (cf. (357)). The mean value and the range for the choline content of dried buttermilk, 231 and 209–259 mg./100 g. (345), are higher than those for dried skim milk ((345), see p. 332) probably for the same reason as that suggested for the higher riboflavin content of buttermilk (cf. (358)).

Sure (359) studied the protein efficiency of mixtures of flour or maize meal with dried buttermilk. The use of buttermilk in poultry rations is discussed in (360,361).

Liquid buttermilk contained copper to the extent of 13–28 μg./100 g. (211). For sixteen samples of Montreal buttermilk mean values for calcium and phosphorus of 117 mg. and 93 mg./100 g. are given in (197).

(*b*) *Dried and evaporated milk*

(i) *General*

Information about evaporated milk with detailed statistics is given in publications of the Evaporated Milk Association (362,363,364) and information about dried milks is found in (365).

The Federal Specification for evaporated milk will be found in (308). Obach (366) presents tables giving full information about the composition, and instructions for use, of various brands of ordinary and acidified dried milks available in Spain. The use of dried skim milk in bread-making is discussed in (341,367,368,369), and paper (370) deals with the nutritive value of such mixtures. Sure (371) describes a new dairy food based on dry skim milk supplemented with yeast, minerals and vitamins A and D.

The 'Average American Diet', based on consumption figures and thus containing a proportion of dairy products, proved unsatisfactory for rats and chicks. The nature of the defects, which led among others to frequent sterility of female rats, remains to be determined (372). Sengupta & Howie (373) found that mice resisted tuberculosis better on a diet containing cereals, meat-and-bone meal, yeast, cod-liver oil and salt, and 14% dried skim milk, than on one of 33% dried whole milk, 66% whole wheat and 1% salt. Addition of meat improved growth, reproduction and lactation of rats on similar milk-wheat-salt diets (374). With three strains of mice, but not with a fourth, improvement in lactational performance resulted from replacement of 'Purina Laboratory

Chow' by a diet containing yeast, casein, dextrin, salts, 'Ruffex', a liver preparation and 50% dried whole milk(375). Branton, Bratton & Salisbury(376) studied the semen production and fertility of bulls on diets containing various concentrate mixtures including skim milk powder. Sexual maturity was delayed in cockerels and pullets given a diet of mineralized whole milk powder supplemented with vitamins(377). The inadequacy for rabbits of an exclusive diet of mineralized whole milk powder is discussed in (378).

(ii) *Vitamins*

Vitamin A and carotene. For the total vitamin A activity of concentrated forms of milk, mainly of European and American brands, the following values (in i.u./100 ml. reconstituted milk) are given from Singapore(91): whole milk powder 48–136, sweetened condensed milk 21–63, evaporated milk 43–118. Russian values(88) for seventy-five samples of dried milk were: carotene 5–333 μg./100 g., vitamin A 0–4 mg./100 g. (see p. 320).

Vitamin B_1. Hodson(379) studied the effect of the heat treatment immediately before use on infant feeds made from evaporated milk to which vitamin B_1 and ascorbic acid had been added to increase accuracy of measurement. The loss of vitamin B_1 was only 9% on bringing the feed to 100° C. in the course of 10 min. or on heating for 8–10 min. at 110° C. Scones made with evaporated or dried milk retained rather less vitamin B_1 after baking than those made with fresh milk(380,381). Guerrant & Fardig(382) studied the contribution of 6% added dried skim milk to the vitamin B_1 content of different types of bread.

Riboflavin. The Australian values(383) for riboflavin, in mg./100 g., were: for six samples each of spray-dried skim milk, Australian 1·76–2·22, American 1·58–1·85; for four samples each of roller-dried skim milk, Australian 1·97–2·31, American 1·46–2·79. Russian values(133) for thirty-four samples of reconstituted dried milk were 48–80 (mean 55) μg./100 ml. For American evaporated milk of not less than 29·5% total solids a value of 510 μg./100 ml. was obtained by biological assay(384). The authors point out that this rather high value might have resulted from deficiencies other than of riboflavin in the test diet used. The work of Guerrant & Fardig(382), just quoted, dealt also with riboflavin. The fluorescence of various fractions of milk was not altered in the course of drying(132).

Choline. The mean value and range for seven samples of dried skim milk are given as 170 and 156–186 mg./100 g.(345).

Vitamin B_{12}. Vitamin B_{12} is present in milk powder(385), but only in relatively small quantities according to (386), and to the extent of 2·5 μg./100 g. according to (387).

Unidentified members of the vitamin B_2 complex. Further reports on the use of dried skim milk diets in the production of 'wrist-stiffness' in guinea-pigs have appeared from the laboratory in which the condition was first noted(388,389), but work from another laboratory(390) confirms earlier failures (cf.(1)) with such diets. The xanthine-oxidase factor ((157), cf. p. 323) is also present in whole milk powder. Whole milk powder, but not dried whey, is said to contain a methanol-extractable factor, present in liver, required by the mink(387).

Vitamin C. Maxa(278) found little loss in actual drying, but a loss of some 40% in the manufacture of sweetened condensed milk. Randoin & Perroteau(160) found relatively less vitamin C in sweetened condensed and evaporated milk than in dried milk. In Hodson's experiments ((379), see above), and in the similar ones of Keeney, Baur & Garrett(314), the immediate losses of vitamin C in the heat treatment of infant feeds were surprisingly small. In subsequent storage, especially in the presence of copper, more marked losses occurred; iron and chlorine as present in treated water had no accelerating effect(314). Thaler(391) gives a detailed summary of a dissertation on the vitamin C content of infant foods, and points out that only the reduced form of the vitamin was measured.

Ascorbic acid added to milk before drying in quantities up to 20 mg./l. increased the keeping quality of the dried product, but total removal by hydrogen peroxide of the ascorbic acid originally present had no such effect ((392), cf. p. 324). Spray-drying increases the capacity of milk to reduce ferricyanide(179).

Vitamin E. For evaporated milk a value of 0·3 mg./100 g. is quoted in(193).

(iii) *Minerals*

Retention by growing rats of over 90% of the calcium from evaporated (393) and from whole dried milk (394) has been reported. The efficiency was greater with milk than with bone meal or calcium carbonate (394).

Values for iron in dried milk are given in (196, 202), the range for six American samples in the latter publication being 500–700 μg./100 g. For copper and lead 240–250 and 120–250 μg./100 g. are quoted from Germany (212); for zinc in whole dried milk 1·30–2·80 mg./100 g. from Germany (212) and 2·5 mg./100 g. from Switzerland (395), and for zinc in evaporated Swiss milk 1·0 mg./100 g. (395).

(iv) *Proteins*

Probably because of ease of handling, more information has appeared about the amino-acid content and composition of dried milk than for liquid milk, but no doubt values for the freshly and properly dried product reflect faithfully the composition of the original milk. Analytical data, mostly by microbiological assay, are available for arginine (396, 397, 398), histidine (397, 399), lysine (397, 400), tryptophan (398, 401), phenylalanine (353, 402), methionine (403, 404, 405), threonine (397), leucine (353, 406), isoleucine (353, 407) and valine (353, 408). An analysis of evaporated milk for seventeen amino-acids is also available (409).

Melnick & Oser (410) in a critical discussion of the influence of heat on the nutritive properties of protein give much valuable information about milk proteins. Hodson (379) found no loss of lysine in the final heat treatment of infant feeds made from evaporated milk.

Work on the inactivation of lysine in stored milk powder through a Maillard reaction was discussed in the previous review (1). Since then further papers have appeared dealing specifically with the reaction (411, 412, 413, 414, 415, 416, 417) and with the changes in the milk protein during storage (409, 418, 419, 420). For inactivation of lysine only moisture but not oxygen is needed. The more marked loss in biological value on storage of dried skim milk in air pack than in gas pack is due to loss of histidine (418, 419, 420). Comparison of the amino-acid content of evaporated milk kept for 5 years with that of freshly prepared samples indicated that losses of lysine, histidine and arginine had occurred during storage (409).

The following values for the proteins of dried skim milk have been reported: a biological value of 78·5 and a true digestibility of 96·6 with growing rats at a 10% level of intake (421), and a biological value of 86 measured by a method designed for adult rats (422); a protein efficiency ratio of 2·30 at a 10% level of protein (423) and ratios of 3·55, 3·30 and 2·91 with protein levels of 7·5, 10·0 and 12·5% (424).

Several papers have dealt with the value of the proteins of mixtures of milk with other foodstuffs. Added to flour of 72% extraction, 1 or 3% of skim milk had no effect on the biological value of the bread proteins but improved the efficiency of food utilization. In a fuller report (425) of work already reviewed (1), and in a further paper (358), Sure discusses the value of dried skim milk in supplementing the proteins of maize meal and wheat flour. The improvement by dried skim milk of the proteins of a poor American urban diet (336) and of a Puerto Rican modified rural diet (426) has been studied.

The relation of dietary proteins, including those of milk, to susceptibility of rats to trauma has been studied by Toby & Noble (427, 428).

The drying of milk had no effect on the fluorescence in ultra-violet light of various milk fractions ((132), cf. p. 322).

The reducing systems developed by heat treatment in dried whole milk and their relation to keeping quality have been investigated (429).

(c) *Ice cream*

The nutritive value of American (430, 431) and of Canadian (432) ice cream has been reviewed. The loss of vitamin C in freezing is negligible and nil during storage for 7 days (278). The vitamin E content of an American sample is given as 0·3 mg./100 g. (193). Canadian values for calcium and phosphorus are 164 and 134 mg./100 g. (197). The browning (Maillard) reaction in ice-cream-mix powder was studied by Tarassuk & Jack (415).

(*d*) *Chocolate milk*

The manufacture of this beverage is given in (433). Experiments with rats indicate no lowering of the availability of milk riboflavin by the chocolate (434). Canadian chocolate drink contained, in 100 g., 113 mg. calcium and 94 mg. phosphorus (197).

(*e*) *Casein, lactalbumin and lactoglobulin*

(i) *General*

The use of casein and lactalbumin in bread-making is considered in (341).

As convenient sources of protein, casein and, to a much less extent, lactalbumin have been extensively used in numerous experiments, but those dealing with the non-specific use of these proteins are not considered here. Casein has been compared with soya-bean protein (435) and beef protein (38,374) or considered as an addition to diets of natural constituents (436) in experiments on reproduction and lactation in rats. Its comparison with other proteins in the effect on susceptibility of rats to trauma is discussed in (427,428).

In studies of the interrelationship between nicotinic acid and tryptophan the effect of the level (437,438,439,440) of casein, of its limiting amino-acids (438,441,442) and of availability (437) or removal (439,443) of its tryptophan was investigated. Paper (444) denies the existence of the interrelationship on diets containing casein.

The synthesis of vitamin C by rats was increased when the amount of casein in the diet was raised from 5 to 18%, apparently through the increased supply of cystine and methionine (445). With adequate casein in the diet, additions of B-vitamins other than riboflavin were without effect on the excretion of vitamin C (446).

The methionine and cystine content of eggs was increased when the low protein diet of the hens was supplemented with 20% casein (447).

Pancreatic proteinase was more efficient than pepsin-HCl in splitting casein *in vitro* (448,449).

(ii) *Protein*

de Vries (450) has reviewed work on the amino-acid composition and nutritive value of casein and lactalbumin. Publication (451) deals exhaustively with all available methods of amino-acid analysis and includes extensive information about the composition of separate milk proteins. From Snell's laboratory comes the description of an ordinary method (452) and of a micro method (453) of microbiological assay of amino-acids in which analyses of casein and colostrum pseudoglobulin for fourteen acids are given.

Analytical data for separate amino-acids in casein (*c*) or lactalbumin (*l*) are given in the papers listed below:
arginine ((396) *c*, *l*; (397) *c*; (454) *l*), histidine ((397) *c*; (399) *c*, *l*; (454) *l*), lysine ((397) *c*; (400) *c*, *l*; (454) *l*; (455) *c*, *l*), tyrosine ((454) *l*), tryptophan ((401) *c*), phenylalanine ((402) *c*, *l*), cystine ((454) *l*), methionine ((226) *c*, *l*;* (403) *c*; (404) *c*, *l*; (405) *c*, *l*), threonine ((397) *c*), leucine ((406) *c*), isoleucine ((407) *c*, *l*; (456) *c*, *l*), valine ((408) *c*), glycine ((457) *c*), aspartic acid ((458) *c*), alanine ((459) *c*), serine ((460) *c*). No hydroxylysine was found in casein (461). The amino-acid composition of α- and β-casein is discussed in (462). A complete analysis by chromatography on starch of amino-acids of lactoglobulin will be found in (463), and values by paper chromatography for aspartic and glutamic acids, lysine, alanine and leucine with isoleucine in (464).

Biological values and true digestibilities recorded for casein by the balance-sheet method are 66 and 99 (465), 80 and 101 (466), 79 and 84 (467) and 72 (biological value) (426), all with growing rats at an 8–10% level of protein intake, and biological values of 63 and 59 at a 20% level of intake (468). With adult rats Mitchell & Beadles (469) obtained a value of 96 for true digestibility but the low biological value of 51, due in their opinion to a relative deficiency of casein in cystine and methionine and to the high requirement of the adult rat for them. Values by the growth method for protein efficiency are: 1·8 at an 8% level of protein intake (426); 2·0 (465), 2·5 (470), 2·2 (471) and 1·7–2·2 (472) with 10% casein in the diet; 2·7 (473) and 1·9 (471) with 18%; 2·4 and 2·6 (468) and 1·9 (474)

* Also β-lactoglobulin.

with 20%. For casein supplemented with methionine a biological value of 89 at a 10% level (475) and a protein efficiency of 3·0 with 18% casein (473) are reported. Whether or not methionine was added, a trypsin inhibitor from soya-bean depressed the protein efficiency of casein (473). The efficiency was enhanced by α-tocopherol and xanthine (472). The effects of added cystine on the biological value and protein efficiency of casein are given in (468). Information on improvement in nutritive value of casein through additions of glycine, arginine or methionine is given in (476), and on the effect of heating and addition of lysine and methionine in (477, 478). According to (479) methionine in intact casein is not readily available to the rat. The biological value and protein efficiency of casein are depressed by deficiency of vitamins of the B complex (468, 475). This is not necessarily a specific effect but probably an expression of the general dependence of normal metabolic exchange upon the availability of any one of the indispensable food ingredients (cf. (480)).

Further assessments of the value of casein for rats (and mice in paper (481)) are given by a repletion method (482), by liver-protein regeneration methods (483, 484, 485, 486), by its effect on blood formation (487) and on the excretion of amino-acids in the urine (481). Nitrogen-balance experiments with dogs are described in (488); the efficiency of utilization of casein is increased by addition of cystine and still further by methionine.

For lactalbumin, forming 11% of the diet, a protein efficiency value of 3·3 for rats is reported (470). By the rat-repletion method (482), from growth experiments with rats (343), from nitrogen-balance experiments with dogs (488) and by measurement of excretion of amino-acids by rats and mice (481) it also proved superior to casein. However, in its ability to regenerate plasma proteins lactalbumin was better than casein for formation of albumin but less efficient for globulin (489). Lactalbumin did not differ from casein in promoting the growth of hypophysectomized rats (490) and was inferior to it for the growth of rats when both proteins were given as 78% of the diet (491).

The effect of heat on the value of casein, and especially the reduction in availability of lysine, is discussed in (410, 492). The value of lactalbumin was also decreased by heat, moist heat being more destructive than dry (493, 494, 495, 496), the damage being probably connected with reduced availability of amino-acids (but lysine was not involved), rather than due to destruction (493). The lactose present as an impurity precipitated the changes (495).

The relation between the lipotropic action of casein and its methionine content is considered in (497).

The porphyrinurogenic factor present in casein treated with hydrogen peroxide is discussed by Dent & Rimington (498).

The assessment of the nutritive value of casein from amino-acid analysis is discussed in (223), and the value of lactalbumin in supplementing poor American diets in (336). Casein gave better retention of nitrogen in dogs than a complete amino-acid mixture, but was less satisfactory for formation of blood protein (499).

Protein-depleted rats gained on the whole more weight when given a mixture of sixteen amino-acids patterned on the composition of casein than from a quantity of casein of the same nitrogen content (500). However, with normal mice casein proved rather better than a mixture of sixteen amino-acids (501), and similar results were obtained with normal rats given a mixture of eighteen amino-acids (502).

The browning reaction between protein and sugar discussed for milk on p. 333 was also studied with casein (411, 412, 414, 503, 504, 505), casein hydrolysate (506) and lactalbumin (496). The biological values for rats of casein (507, 508) and of the hydrolysate (506) were thereby markedly reduced, though the changes in amino-acid content as measured microbiologically were only quite small (506). Apart from lysine, the reaction in the hydrolysate was said to involve histidine, threonine, tryptophan, phenylalanine and methionine (506).

Owing to their increasing therapeutic use, protein hydrolysates have occupied much attention. Papers (509, 510, 511, 512) deal with the amino-acid composition of casein hydrolysates, and paper (507) also with that of lactalbumin.

The value of casein hydrolysates for restoration of weight in protein-depleted rats (513) and for regeneration in rats of liver protein (485) was studied. Tests with dogs and rats showed that

pancreatic digests of casein (514) and those made with bacterial and fungal enzymes (515) had the same protein efficiency as the intact protein. A partial acid hydrolysate proved, however, rather less satisfactory (516). Ågren (517) reported a remarkable improvement in efficiency of food utilization by rats given an enzymic hydrolysate of casein (518) in addition to a stock diet (519), but made no parallel tests with intact casein. Paper (520) deals with the relation of casein hydrolysates to hypertension and nephrosclerosis in rats.

The utilization of casein hydrolysates given intravenously, has been studied with dogs (521, 522, 523) and with rats (524).

Comparison of hydrolysates of casein with those of lactalbumin showed that, as with the proteins themselves, lactalbumin was superior for the more general purposes of growth (489, 507) but not for the regeneration of plasma proteins (489, 507, 525, 526) or blood proteins (527). The value of such hydrolysates is considered in (528, 529), and the effect on the growth of rats of additions to casein hydrolysate of tryptophan and of methionine in (528).

Casein digests are, like amino-acids, readily available sources of nitrogen and carbohydrate (530). An enzymic digest of casein was successfully used by Gustaffson (41) as a component of a rat-milk substitute in the rearing of germ-free rats.

(iii) *Impurities in casein of nutritional significance*

Casein of unstated origin contained 900 μg./100 g. of riboflavin and 700 μg./100 g. of pantothenic acid (531). Values for pantothenic acid in different grades of casein given in (532) were 70–90 for 'Smaco', 130 for 'GBI', 180–200 for ether-extracted, 190–280 for acid-washed and 150–280 μg./100 g. for alcohol-extracted casein.

Of nicotinic acid commercial casein contained 9–12 mg./100 g. (174, 533).

Crude casein contains vitamin B_{12} ((147, 149, 151), see also (378, 534, 535, 536)), according to (537) to the extent of 10·4 μg./100 g., and 'vitamin-free' casein contains 1·7 μg./100 g. (153); exhaustive extraction with hot alcohol is necessary for complete removal of factor X (538, 539) now considered identical with vitamin B_{12} (151). 'Labco' casein contains less 'zoopherin', believed to be the same as factor X, than crude casein (540).

Papers (541, 542, 543) discuss the presence and demonstration of strepogenin in casein, but its value is doubted for the mouse (501) and denied for the rat (502).

Casein is devoid of the factor maintaining the xanthine-oxidase activity in the liver (157, 158). Acid-hydrolysed casein is, however, more active (479).

Casein contains small amounts of the tomato-juice factor for *Lactobacillus lactis* (544). The presence in it is reported of unidentified factors for dogs (526) and rats (545, 546).

(*f*) *Lactose*

Fischer & Sutton (547) have reviewed the effects of lactose on gastro-intestinal motility.

Hens cease laying on purified diets containing lactose as the carbohydrate; addition of fat restores production (548). Dried defatted liver has a beneficial effect on the growth and development of rats receiving diets high in lactose (549).

Neither dry whey nor lactose stimulates the intestinal synthesis of biotin by the fowl (550) but lactose and, to a less extent, galactose promotes it, and also that of riboflavin and folic acid in the cotton rat (551). The part played by intestinal bacteria in the digestion of lactose by the rat is discussed in (552).

Lactose, though it does not undergo fermentation, is as cariogenic for the rat as sucrose and glucose (553).

The relationship between fat and the metabolism of galactose is discussed on p. 327.

IV. NUTRITIVE VALUE OF MILK FOR MAN

A. GENERAL

(*a*) *Introduction*

Though the political horizon is again darkening, the world food situation is, on the whole, easier than at the time of writing of the previous review(1).

The well-documented and informative summaries of the Commonwealth Economic Committee(554,555) show that the world production of milk and dairy produce has been rising slowly (though it is doubtful whether it is keeping pace with increases in population), and further information about them will be found in F.A.O. publications(556,557,557*a*,558,559,560,561) including a new *Monthly Bulletin of Food and Agricultural Statistics*(562). Statements for several countries are also given in (563). Consumption data are given in (554,555,559,560,563). Information about production in the United States will be found in (364,564,565,566), and about consumption in (364,564,565,567,568). The interesting data in the last two publications cover the period 1909–48. Information about production and consumption in the United Kingdom is published monthly by the Central Statistical Office(569); paper(570) should also be consulted; official information is available about consumption levels up to June 1949(571). The wartime changes in sources of supply are given in(572). Canadian food and nutrition statistics including numerous data about the contribution of milk produce are given in (573). Further publications deal with production(574,575,576,577) and consumption(574,575,576,577,578) in Australia, with consumption in Sweden since 1876(579), and with conditions in Finland(580) and Puerto Rico(581). Statistics for Northern Rhodesia will be found in reports of the Veterinary Department(582,583,584,585,586), and for Western Australia in those of the Milk Board(578). Interesting statistics about the supply and consumption of milk in Paris from 1900 to 1947 are quoted in (587). The daily consumption per head of population was 0·293 l. in 1900, reached 0·323 l. in 1910, fell to 0·122 l. in 1942 and 1943 and was 0·170 l. in 1947. More recent values will be found in trimestral reports of the Ministry of Public Health(588). Russian production plans up to 1951 are given in (589). United Nations reports deal with the role of milk in the nutrition of rice-eating communities of south and east Asia(590), with help in the conservation of milk(591) and with the nutrition policy of the F.A.O.(592) and of the U.N.I.C.E.F. food-relief work(593). Another F.A.O. report discusses the better utilization of milk(594), and further suggestions on this question will be found in(18,19,20,21). The use of milk for children in wartime India is considered in (595), and that of powdered milk for children in Europe in (596). Wilder & Keys(597) mention the position of milk among foods for emergencies. Information about the distribution of imported milk in Malaya after re-occupation is given in (598). Spencer(599) discusses the future of milk consumption in the United States, and plans of investigation of milk for the U.S. Forces will be found in(600). Callert(601) describes the functions of the Milk Propaganda Association.

A revised version of the statement on the recommended dietary allowances of the (U.S.A.) National Research Council(602) has appeared. It gives, as before, patterns of diets in which milk and its products figure prominently. The Canadian Council on Nutrition has made a similar pronouncement for Canada(603). The British Medical Association has also announced its dietary recommendations(604). The publication contains valuable information about nutrition in the U.K. Booher & Behan(605) have calculated the nutrient composition of a diet representative of the U.S. food supplies of recent years, including in a 3000 Cal. portion 360 g. fresh whole milk, 26 g. evaporated milk, 10·2 g. dried skim milk and 9·3 g. cheese.

(*b*) *Nutritional surveys*

Again only a small selection from the large number of publications can be quoted here. The F.A.O. has produced a report on the technique of surveys(606); a review of world trends in the last decade covering 75% of the total population indicates that the amount of milk now available per head, calculated as fluid milk equivalent, 200 g. daily, differs little from that available before the war(607). In the United States, where the consumption of milk has been rising since the beginning

of the century (608), surveys were published dealing with children (609) and adults (610) in New York State, with children in institutions (611,612), with freshmen in a college (613), with women from middle to old age (614) and with rural families in North Carolina (615). American soldiers consumed in 1945, on the average, 1·26 lb. daily of fluid milk equivalent as against 1·02 in 1941–3 (616). The wartime changes in consumption of milk, cream and butter in Vermont are discussed in (617). Papers (618,619) deal with the value of milk in American school lunches, and a note on milk in such lunches will be found in ((620), p. 137 there). In Canada the milk consumption by soldiers in a subarctic climate (621) was definitely lower than by the American Army (616), most probably because of shortage of fresh milk. The Newfoundland survey of 1944 (cf. (1)) was repeated in 1948 (622,623).

Information is available from Iceland (624), from Finland for the period 1941–6 (625), and from Holland in the wartime-starvation period (626) and as a summary of surveys carried out between 1916 and 1947 (627). In addition to the regular reports from France on the nutrition of the population already mentioned (588) the same journal summarizes the surveys from 1940 to 1947 (628), and deals with levels of consumption in 1948–9 (629), with the consumption by schoolchildren of chocolate milk (630,631) and with that of milk supplements in orphanages (632). The food budget of a Parisian family will be found in (633). Indian surveys are reported in (634,635), and those done there before the end of 1939 are summarized in (636). Paper (637) deals with boarding and half-boarding schools in Brazil, and paper (638) with a Puerto Rican rural community. In Puerto Rico milk was largely taken with coffee, and adults consumed most, and children least, of the scanty supply.

Data from French West Africa will be found in (639).

The results of the Irish National Nutrition Survey have been published in full (640,641,642,643) and are also available in a summarized version (644,645,646,647,648).

In this country information about the consumption of milk and milk products is given in (649), and statistics for the milk-in-schools scheme in 1946 and 1947 appear in ((620), p. 23 there); close on 90% of all schoolchildren consume, under this scheme, at least one-third of a pint daily.

Magee & Wright (650) have discussed the rising consumption of milk in Great Britain in relation to health. A survey of the food habits of athletes at the last Olympic Games (651) includes information about milk. The economics of milk consumption are considered in (652,653,654,655).

(*c*) *General effects*

Much valuable information about the use of milk in infant feeding will be found in (6*a*). Jeans (22) and Stevenson (23,656) discuss the best use of milk for the artificially fed baby and for children (22), and the need for supplementation with certain vitamins. According to them, despite all modern refinements of artificial feeding, breast feeding remains the ideal procedure. No such superiority is noted in (657). Premature babies do better on cow's milk when extra calcium is given (22). The value of cow's milk for such babies was mentioned in the previous review (cf. (1)); corroborative evidence will be found in (53,658,659). Paper (660) discusses the production of acidosis in premature babies by protein milk. According to (661), however, protein milk as an addition to breast milk proved very suitable for such babies.

In forty-one pairs of malnourished children Spies & Dreizen (662) observed a marked improvement in those given supplements of milk. The diet itself is not stated, and the control children received no other addition. In Malaya, in conditions of food shortage, schoolchildren derived benefit, as measured by gains in height and weight, from the daily addition of 5–6 oz. milk, but milk with a school meal had a much more marked effect (598).

A further statement has appeared (663) about the Toronto inquiry into the influence of the pre-natal diet on mother and child mentioned in (359).

The diets recommended for pregnant and nursing women at the Sagene Health Station in Oslo, and the results obtained, are described in (664), and the use of fresh milk in infant feeding in India in (665).

Waterlow (666) obtained good results with milk in the treatment of fatty liver disease of infants in the British West Indies, a disease possibly related to that observed among the South African Bantu whose diet contains soured milk curds, but not the whey (cf. (1) and p. 329). The protective action of milk in dental caries is discussed by Fosdick (667) and information about it will also be found in (668).

Of various animal foods tested, milk had the most consistent antithyroid effect for human adults(669).

Milk given to adults to replace, calorically, a freely chosen lunch produced neither the feeling of satiety nor the blunting of perception of smell associated with the meal(670). The preference of growing boys in California for milk bread increased gradually with the increase in milk content in three steps from 0 to 14%(671).

The use of lactose in milk mixtures is discussed in (657), and its effect on intestinal motility in (547). Paper (672) deals with the absorption of galactose from the small intestine of the adult. Paper (673) describes a further case of galactosemia in an infant, and paper (674) a case of milk allergy. A detailed consideration of the latter condition in infants based on the study of twenty-three cases in Sweden will be found in (674*a*); its treatment in adults is given in (675). According to American experience(676) milk is less likely to cause asthma in children than fish, eggs, walnuts, peanuts or chocolate.

Favourable tests with children and adults are reported(371) of the new dairy food mentioned on p. 331.

B. MILK COMPONENTS

(*a*) *Vitamins*

In New South Wales, dairy products contribute two-thirds of the vitamin A intake of the population(577). According to Jeans(22) neither breast-fed nor artificially fed babies require additional vitamin A, though Stevenson(23) would like the latter to get as much vitamin A as those breast-fed. The dangers of sweetened condensed milk for babies and its assocation with keratomalacia are emphasized by Williamson & Leong(91).

Dairy products provided 13% in Canada(573) and 11% in New South Wales(577) of the vitamin B_1 intake; the Canadian contribution of riboflavin was 50%(573). The effect of milk intake on the riboflavin metabolism of young women is given in (677).

A boy of 9 whose diet since birth consisted of milk only, of which at the time of observation he was taking 5 pints daily, apparently without addition of vitamin D, had abnormally slender bones with delayed ossification and severe general decalcification(678).

(*b*) *Proteins*

The population of Canada derived 23% of its protein(573), and that of New South Wales 15%(577), from dairy products.

Mean biological values of 69 for cooked and 56 for raw casein were obtained in tests with nine to ten adults(679). Calculations based on the requirements of infants for cystine and methionine, and the amounts of these acids present in milk indicate that breast milk provides a very small margin of safety during the early months of life but that cow's milk is much better in this respect(680). These observations may have a bearing on the relative merits of the two milks for premature babies already discussed on p. 338. According to Rose(681) methionine is an essential amino-acid also for the adult, a statement at variance with observations by other authors discussed in the previous review(1). In view of the growing evidence that vitamin B_{12} is connected with the formation of methionine(682,683) it is of interest that the liver concentrate used by Rose in his tests with amino-acid mixtures may have been devoid of vitamin B_{12}(681).

Rossier & Beauvillain(684), working with infants, obtained better retentions of nitrogen from mother's milk than from cow's milk when both supplied equal amounts.

Leverton & Gram(685) found that women retained nitrogen more satisfactorily when a daily supplement of 24 oz. of milk was evenly distributed between three meals than when the breakfast portion was transferred to the evening meal.

Infants utilized the nitrogen of a synthetic milk, derived from a meat hydrolysate, as efficiently as that of an evaporated milk mixture(686).

Studies in India dealt with the value of curd protein as an addition to a poor South Indian diet(687), and with the addition of milk as a source of protein to predominantly vegetable diets(688,689).

Steele, Reynolds & Baumann (690) studied the appearance of amino-acids in the blood and urine of adults after the consumption of different sources of protein, including milk, and Ross (691) determined amino-acids in the stools of breast fed and bottle fed infants.

The utilization of casein hydrolysates is discussed in (692,693,694), and the value for surgical patients of a high protein derivative of lactalbumin in (695).

Cocoa had no marked effect on the nitrogen retention of young women (696).

(c) *Minerals*

Of the total calcium supplies of Canada some 77 % are said to have come from dairy products (573); the figure for New South Wales is 76 % (577).

Several papers have appeared dealing with the metabolism of calcium, largely derived from milk, in children (697,698,699), and in adults (394,689,696,700,701,702). From diets supplying daily 1 g. or more of calcium the children retained about 20 (697) to 26 % (699). When the intake was cut down from 1 to 0·5 g. the retention fell to 8 % (697). Similarly, low retentions were observed with children, in negative calcium balance on a diet supplying about 0·2 g. calcium daily, when this was supplemented by about 0·36 g. calcium from milk (698).

Calcium was equally well retained from bone meal (394), soya-bean milk supplemented with dicalcium phosphate (698), buffalo milk (702) and, by a pregnant woman, from pumpkin leaves (689), as from cow's milk. For two adult males the pumpkin leaves were a more efficient source than milk (689). Addition of cocoa had no significant effect on the calcium balance of eight young women, but there was a significant shift of excretion from urine to faeces (696).

The question of adaptation to low intakes of calcium has again been considered. The impressive nature of the evidence is acknowledged by a committee of the British Medical Association (604). Kraut & Weckel (700) have published further findings supporting their earlier data (cf. (1)). The two subjects in the experiments of De & Som (354), though in negative balance with a diet containing 0·25 g. calcium from vegetable sources, changed to positive balance when whey supplied an additional 0·139 g.

Dairy products are said to have supplied 3 % of the iron intake in Canada (573) and some 5 % in New South Wales (577). According to (703) 200 ml. milk reduced the absorption of iron by adolescent boys to the same extent as 285 g. cooked rolled oats. In a study of rickets in the Department of Corrize in central France its prevalence there is ascribed, among others, to the abnormally low content in the local milk of calcium and phosphorus (704). It seems more probable, however, that another of the stated causes, lack of vitamin D and of exposure to sunlight, is in itself sufficient to account for the disorder.

(d) *Fat*

Basu (689) has compared, on four adults, the digestibility of buffalo and cow butterfat with that of vegetable fats and oils. Except for vanaspati (see p. 327) the digestibilities were between 94 and 99 %. Some 160 American children in one institution using, by design, margarine as the only table and cooking fat, made the same physical progress as 107 children in another institution where butter was similarly used (705).

C. EFFECT OF TREATMENT

(a) *Effect of heating and processing*

Papers (660,706,707) may be added to the long list of those dealing with the excellent record of evaporated milk in infant feeding; they underline its value in mixtures not only for full-term but also for premature babies.

According to (657) the sugar-tolerance curve with raw milk is similar to that with raw mother's milk, but the shape of the curve is altered by pasteurization or evaporation. According to the authors, the evidence is insufficient to determine whether raw milk would be superior to heated in artificial mixtures. The use of evaporated milk for older children is discussed in (708,709).

The availability of riboflavin in ice-cream was almost as high as that of the pure vitamin (710).

(*b*) *Irradiated and vitaminized milk* (*all types*)

According to Weckel(315) skim milk supplemented with vitamins A and D should be a valuable food for over-weight and old people and for those suffering from coeliac disease, and for special diets.

The use of vitamin D milk for infants and children is discussed by Jeans(310).

In Germany, Scheer, the protagonist of irradiated milk, has supplied further evidence for the effectiveness of his method of treatment of rickets(711,712,713,714), and a supporting contribution comes from Graser(715). The special value of irradiation has, however, been challenged in Germany in a discussion(716) and in articles(299,300,301,302) which favour the American practice of adding vitamin D to milk, but to the extent of 1000 i.u./l.

In this country, good results with evaporated milk fortified with 500 i.u. vitamin D_3 to the reconstituted quart are reported by O'Brien & Smellie(311).

The special evaporated milk supplemented with minerals and vitamins, developed by McCollum & Grubb, and mentioned in the previous review(1), has now been put to clinical tests(717,718) in which it proved its worth.

(*c*) *Soured milk*

Hesse(719) has reviewed the information about whey beverages, Meulemans(720) and Mégevand & von Riederer(721) the value of acid milk mixtures in infant feeding, and Fischer & Sutton(547) have dealt with the effect of soured milk on intestinal motility. Good results with children suffering from diarrhoea and intestinal disorders are reported when buttermilk is used(355,722). Olevskiy(317) found higher acidity in the stomach contents of children after kefir than after fresh milk. Yoghurt bacteria do not become established in the intestine(723,724), but the effects of yoghurt may nevertheless be favourable, owing to its content of lactose and lactic acid(724).

In Batavia, immediately after the Japanese war, anaerobic lactic-acid fermentation of reconstituted dried milk gave a most valuable product for the treatment of malnourished infants(725). Xerophthalmia, curable by vitamin A, developed in two infants given an exclusive diet of buttermilk for 6 months(355).

Joshi(726) is of the opinion that dahi (curds) should not be given to patients under sulphonamide treatment, since *p*-aminobenzoic acid synthesized in it by *Torula dahi* might antagonize the bacteriostatic action.

According to Roine & Porvari(701) souring has no effect on the availability of calcium in milk, but Desikachar & Subrahmanyan(702) are of the opinion that it exerts a favourable effect.

De & Som(354) attribute the remarkably high retentions of calcium from whey, observed by them in two adults, to the effect of lactic acid, but adduce no proof by comparison with fresh milk.

(*d*) *Soft-curd milk*

Spur discusses the definition of soft-curd milk (727) and its digestibility(728), and Trout has reviewed the nutritive value of homogenized milk(325).

We should be grateful to authors for reprints of articles dealing with topics reviewed by us, especially those appearing in journals not normally connected with dairying.

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